UNIVERSITY OF ALBERTA

Biosynthetic Studies on Dehydrocurvularin, and Modification of the Swern Oxidation

by

Yaoquan Liu

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

DEPARTMENT OF CHEMISTRY

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To my wife, Ruiying, and daughter, Alice

ABSTRACT

The biosynthesis of dehydrocurvularin (**1**) and 8-hydroxycurvularin (**3**) from *Alternaria cinerariae* ATCC 11784 was examined by incorporating advanced precursors and blocking post-PKS transformations. *N*-Acetylcysteamine (NAC) thioesters of [2,3-^{13}C$_2$]-acetoacetate (**7**), (*S*)-[2,3-^{13}C$_2$]-5-hydroxyhexanoate (**8**) and (*S*)-[2,3-^{13}C$_2$]-7-hydroxyoctanoate (**27**) were synthesized and fed to the culture of *A. cinerariae*, in conjuction with β-oxidation inhibitors. Incorporation results provide important information about the structures of the PKS enzyme-bound intermediates. Using P-450 inhibitors to block the post-PKS oxidative processes resulted in no accumulation of any metabolites less-oxidized than **1**, suggesting that **1** is the initial product released from the PKS. Investigations on the late stage transformations reveal an equilibrium between **1** and **3**, catalyzed by some component(s) in the medium (potato dextrose broth).

A number of methodologies were used in attempts to synthesize the possible pentaketide intermediate NAC (*S*)-[2,3-^{13}C$_2$]-9-hydroxy-3-oxodec-4-enoate (**50**). The ethyl ester can be constructed by Horner-Emmons reaction, but NAC ester **50** is not easily accessible due to the presence of sensitive functional groups and the facile lactonization of intermediates.

N-(3-Hydroxypropionyl)cysteamine (**28**) was designed as a substitute of NAC, having an extra hydroxyl functionality for attachment of other molecules such as sugars to achieve active transport through the cell membranes. Feeding of (*S*)-[2,3-^{13}C$_2$]-7-hydroxyoct-2-enoic acid, as the thioester of **28** failed to show any intact incorporation, which suggests that **28** is not accepted by the PKS machinery.

The Swern oxidation, a widely-used synthetic procedure that converts alcohols to aldehydes or ketones, was modified to eliminate the odorous by-product, dimethyl sulfide. 6-(Methylsulfinyl)hexanoic acid (**98**) was found to be a successful substitute for DMSO in

the Swern oxidation. The by-product 6-(methylthio)hexanoic acid (**97**) is non-volatile, can be readily separated from the product mixture and recycled with sodium metaperiodate. The mechanism of this modified process was studied with low temperature (-60 °C) ^{13}C NMR spectrometry.

The use of polymer-supported sulfoxide reagents was explored. Loading of dimsyl sodium, sodium thiomethoxide (subsequently oxidized), sodium 6-(methylsulfinyl)-hexanoate and sodium 6-(methylsulfinyl)hexoxide onto chloromethyl polystyrene resin generates polymeric sulfoxide reagents **114**, **119**, **125** and **126**, respectively. All of these polymers work efficiently in the Swern oxidation, but lose oxidation capacity upon regeneration after the Swern reaction.

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LIST OF ABBREVIATIONS

$[\alpha]$	specific rotation
Ac	acetyl
ACP	acyl carrier protein
anhyd	anhydrous
APT	attached proton test
Ar	aryl
AT	acyl transferase
bp	boiling point
br	broad
Bu	butyl
n-BuLi	*n*-butyl lithium
calcd	calculated
CDI	carbonyl diimidazole
CI	chemical ionization
CoA	coenzyme A
concd	concentrated
δ	chemical shift in parts per million downfield from TMS
d	doublet
DCC	1,3-dicyclohexylcarbodiimide
DEBS	6-deoxyerythronolide B synthase
DIBAL	diisobutylaluminum hydride
DH	dehydratase
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide

DMSO	dimethylsulfoxide
DPPA	diphenylphosphoryl azide
EI	electron impact ionization
Enz	enzyme
ER	enoyl reductase
Et	ethyl
FAB	fast atom bombardment
FAS	fatty acid synthase
HOBT	1-hydroxybenzotriazole
HPLC	high performance liquid chromatography
IR	infrared
J	coupling constant
KR	ketoreductase
KS	ketoacyl synthase
LHMDS	lithium hexamethyldisilazane
m	multiplet
m/z	mass to charge ratio
MCPBA	*m*-chloroperbenzoic acid
Me	methyl
MeO-PEG	polyethylene glycol monomethyl ether
MHz	megahertz
mp	melting point
MS	mass spectrometry
NAC	*N*-acetylcysteamine
NCS	*N*-chlorosuccinimide
NMR	nuclear magnetic resonance
Nu	nucleophile

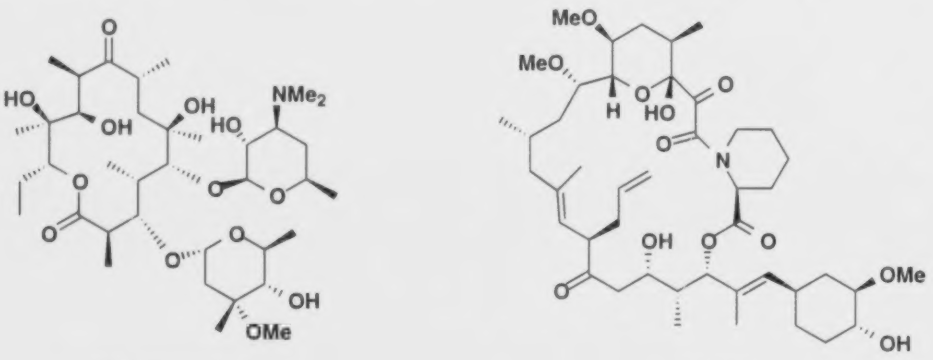
ORF	open reading frame
PDB	potato dextrose broth
Ph	phenyl
PKS	polyketide synthase
ppm	parts per million
Pr	propyl
Pyr	pyridine
q	quartet
RD	relaxation delay
Rf	retention factor
RP	reverse phase
rt	room temperature
s	singlet
SAM	*S*-adenosyl-L-methionine
t	triplet
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
TE	thioesterase
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane
Ts	*p*-toluenesulfonyl
UV	ultraviolet

Chapter 1 Biosynthetic Studies on Dehydrocurvularin

INTRODUCTION

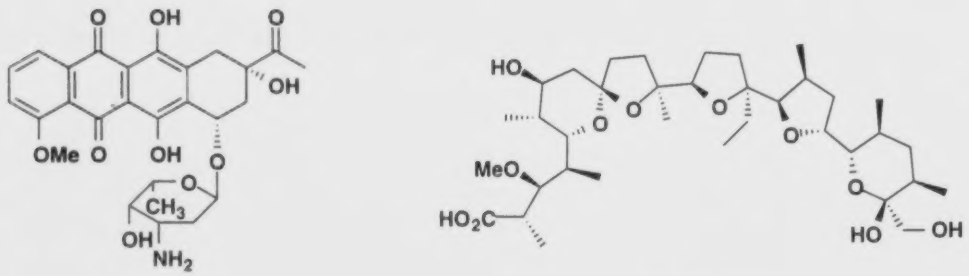
1. Biosynthesis of Polyketide Metabolites

Polyketides are a large family of structurally diverse natural products, most of which are produced by Actinomycete bacteria and fungi.[1] They are an extremely rich source of biologically active molecules. Some notable examples include antibiotics (such as tetracyclines and erythromycin), anticancer agents (daunorubicin), immunosuppressants (FK 506 and rapamycin), and veterinary products (monensin and avermectin) (Figure 1).



Erythromycin A

FK 506

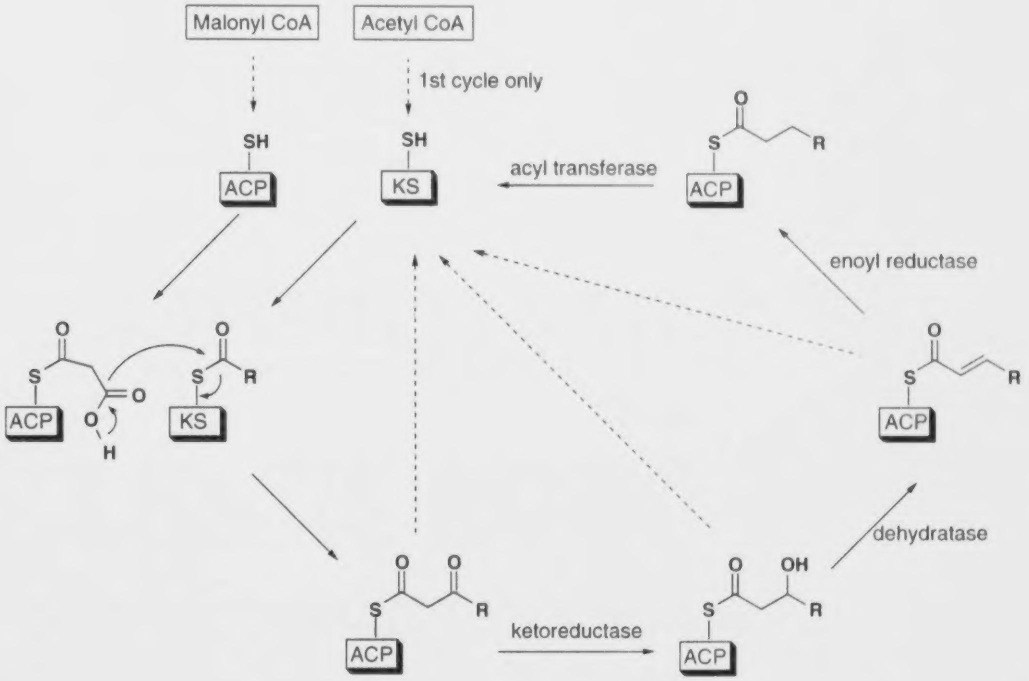


Daunorubicin

Monensin A

Figure 1 Structures of some polyketide metabolites

Despite their structural diversity, polyketides are thought to be closely related biosynthetically in the early steps when the core of the structure is assembled. Biosynthetic and molecular genetic studies have demonstrated that these basic structures are synthesized by multifunctional polyketide synthase (PKS) enzymes, which catalyze repeated condensation cycles between acyl thioesters in a fashion similar to fatty acid biosynthesis.[2] Each condensation cycle results in the formation of a β-keto group on a growing carbon chain that may then undergo none, some, or all of a series of reductive steps (Scheme 1).

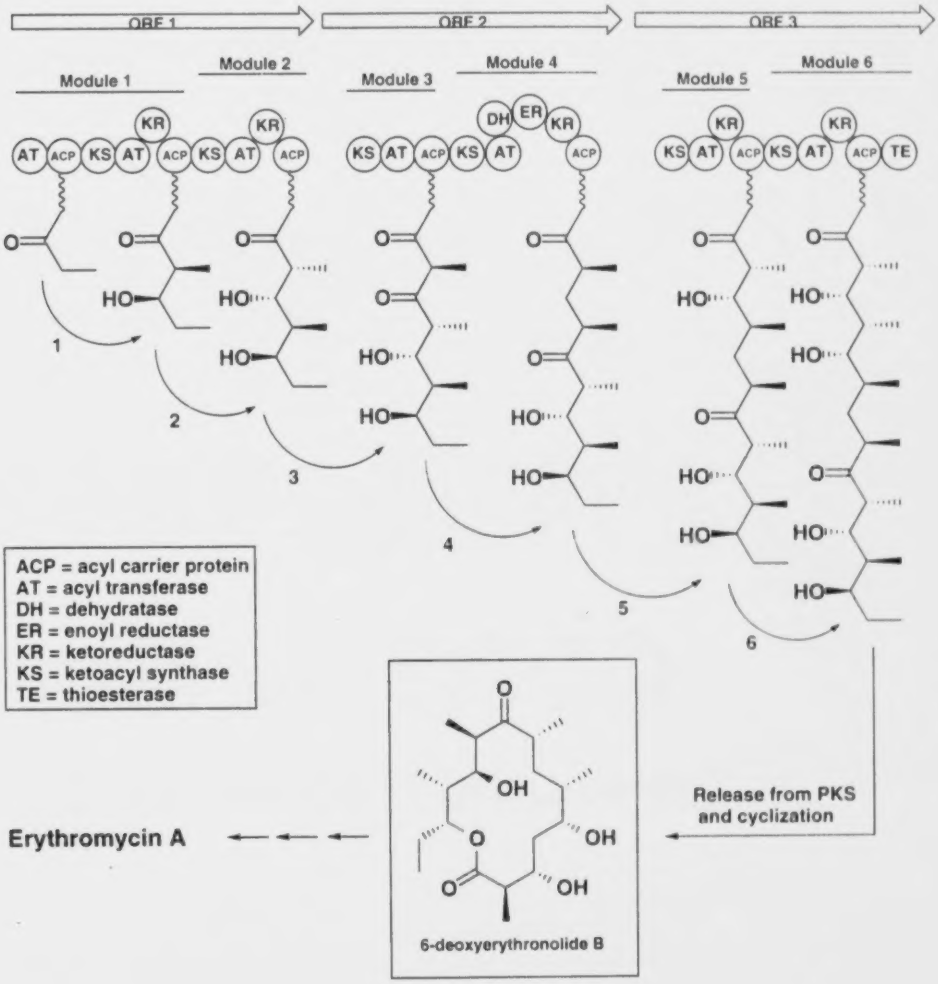

Malonyl CoA
Acetyl CoA
1st cycle only
SH
ACP
SH
KS
acyl transferase
enoyl reductase
dehydratase
ketoreductase
S
R
O
OH
H

Scheme 1 Fatty acid and polyketide synthesis

Initially, the chain starter (commonly acetate) on the ketoacyl synthase (KS) condenses to an extender unit (malonate) on the acyl carrier protein (ACP) with concomitant decarboxylation. Fatty acid synthases (FAS) generally reduce the resulting β-ketothioester of ACP to a saturated chain in a series of steps, namely, β-keto reduction, dehydration and enoyl reduction. This is then transferred to the keto synthase protein for the next condensation with a new extender unit. However, the polyketide synthases can short cut this cycle (dashed arrows in the middle) by acyl transfer to the keto synthase for the addition of the next unit before the reduction sequence is complete. This leads to incorporation of keto, hydroxyl, or olefinic functionality in the polyketide chain as it grows. Also, unlike FAS, the more highly programmed PKS can utilize a large range of starter units (e.g. linear or branched carboxylic acids) and more extender units (usually malonyl, methylmalonyl, or ethylmalonyl). The variation in the oxidation level of the growing polyketide chain and the variety of both starter and extender units serve to introduce immense structural diversity.

The intermediates of the carbon-chain assembly are thought to remain enzyme-bound as thioesters of a protein thiol group until the carbon chain reaches its full length. This hypothesis is based on the principles that have emerged from work with purified FAS from many organisms.[3] The inhibition of polyketide synthesis by thiol-specific reagents such as cerulenin also supports this idea.[4] After release from the assembly enzymes, the products are further processed by the post-PKS enzymes to form the final metabolites. These post-PKS transformations may include P-450 oxidation, methylation by *S*-adenosylmethionine (SAM) and glycosylation, and add yet another level of diversity.[2b]

The application of molecular genetics to polyketide biosynthesis provides valuable insights into the organization and function of the PKS.[5] Cloning and sequencing of PKS genes has shown two entirely different programming strategies. One is represented by the PKS enzymes for macrolides, such as the clinically important antibiotic erythromycin A.[6] This system consists of several large multifunctional proteins carrying a set of separate active sites for every individual step of carbon chain assembly and modification. As shown in Scheme 2, erythromycin A is derived from propionyl-CoA and six molecules of (2*S*)-methyl-malonyl-CoA through the sequential action of such sets of enzymes housed in the three multienzyme polypeptides of 6-deoxyerythronolide B synthase (DEBS). The second class of PKS enzymes, represented by the synthases for aromatic compounds, has a single set of iteratively used active sites.[7] The programming mechanism for this class of PKS is not apparent from the number and rearrangement of active sites.

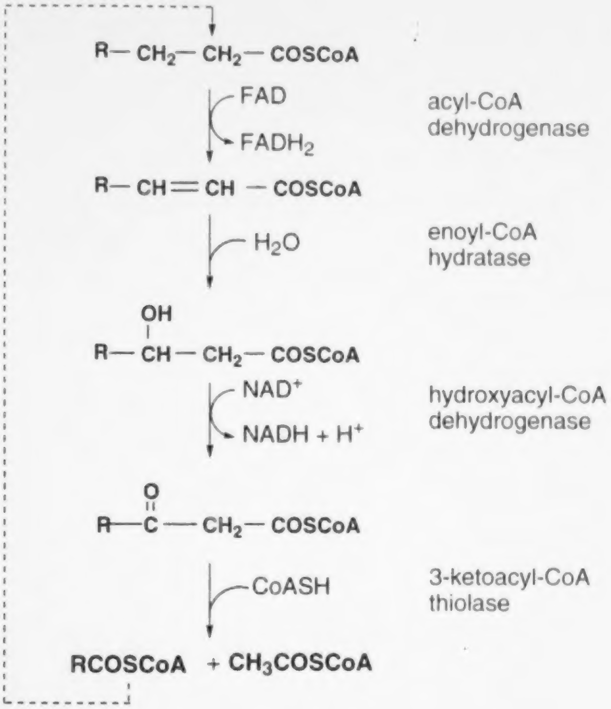

ORF 1
ORF 2
ORF 3
Module 1
Module 2
Module 3
Module 4
Module 5
Module 6
AT ACP KS AT KR ACP KS AT KR ACP
KS AT ACP KS AT DH ER KR ACP
KS AT KR ACP KS AT KR ACP TE
1
2
3
4
5
6
ACP = acyl carrier protein
AT = acyl transferase
DH = dehydratase
ER = enoyl reductase
KR = ketoreductase
KS = ketoacyl synthase
TE = thioesterase
Release from PKS and cyclization
Erythromycin A
6-deoxyerythronolide B

Scheme 2

Most of the biosynthetic information about the polyketides has been obtained after incorporation of isotopically enriched precursors into the metabolites during their biosynthesis. Early studies in the late 1950s and early 1960s utilized radioactively labeled precursors.[8] The metabolites were chemically degraded to isolate individual carbon atoms, such that the specific location of the radioactivity could be detected. These studies were both labor intensive and time consuming. With the rapid development of nuclear magnetic resonance (NMR) technology over the last twenty years, a new approach using stable isotopes has been widely applied to biosynthetic studies.[9] This provides information about regiospecific incorporation directly.

Numerous experiments have shown that suitable precursor compounds with proper stereochemistry, activated as *N*-acetylcysteamine (NAC) derivatives, can be incorporated into the final metabolites.[10] The NAC thioester moiety is essential for intact incorporation, and is believed to mimic the acyl-ACP thioester in the active site of the polyketide synthase.[10j] These results support the notion that the oxidation level and stereochemistry of the growing polyketide chain are usually adjusted prior to each step of chain elongation.

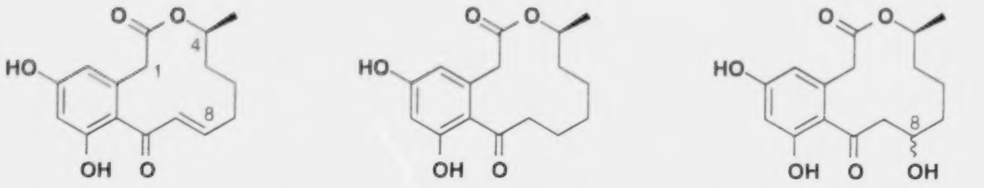
A major problem encountered with attempted loading of advanced precursors onto the polyketide synthase is the competitive degradation of these compounds by highly effective β-oxidation enzymes. β-Oxidation is a common pathway for the degradation of saturated and unsaturated fatty acids in eukaryotes and prokaryotes.[11] The degradation involves the successive oxidative removal of acetyl groups from the carboxylic end of the long chain fatty acids as shown in Scheme 3. It thus cleaves the labeled precursors into small carboxylic acids before their incorporation into the metabolite, thus severely decreasing the amount of the intact utilization. Fortunately, β-oxidation inhibitors have been successfully used to suppress this undesired process.[10e] It is also known that high glucose concentrations can partially suppress the β-oxidation pathway in mammalian systems since their acetate requirements can be met by catabolism of the carbohydrates.[12]


R— CH2— CH2— COSCoA
FAD
FADH2
acyl-CoA dehydrogenase
R— CH═CH — COSCoA
H2O
enoyl-CoA hydratase
OH
R— CH — CH2— COSCoA
NAD+
NADH + H+
hydroxyacyl-CoA dehydrogenase
O
R—C — CH2— COSCoA
CoASH
3-ketoacyl-CoA thiolase
RCOSCoA + CH3COSCoA

Scheme 3

2. Dehydrocurvularin, a Macrolide Phytotoxin

Dehydrocurvularin (**1**) and its closely-related family members, curvularin (**2**) and 8-hydroxycurvularin (**3**) (Figure 2), are macrolide phytotoxins produced by a number of fungal species, especially members of *Curvularia*,[13] *Penicillium*[14] and *Alternaria*.[15] All of these metabolites show potent cytotoxic activity towards sea urchin embryogenesis and block cell division at low concentration (1.2 µg/mL for **1**, 2.5 µg/mL for **2**, 50 µg/mL for **3**).[14a]



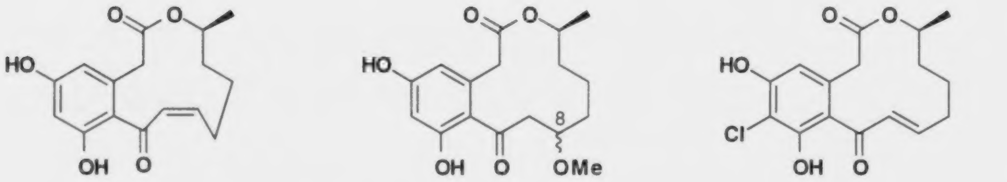
Dehydrocurvularin **1**

Curvularin **2**

8-Hydroxycurvularin **3**

Figure 2 Structures of dehydrocurvularin, curvularin and 8-hydroxycurvularin

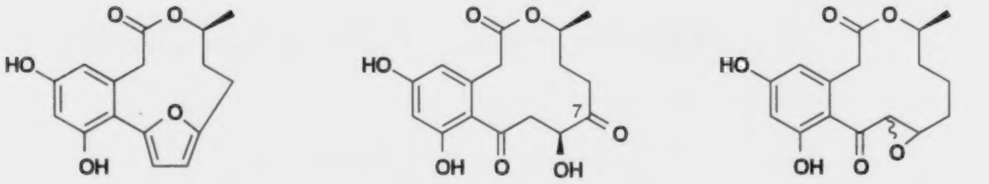
Dehydrocurvularin was first isolated from the culture filtrate of a strain of *Curvularia* by Musgrave in 1956.[13a] Its structure was elucidated by Munro *et al.*[13b] using spectroscopic analysis as well as the chemical conversion to curvularin. Its co-occurrence with curvularin and 8-hydroxycurvularin has been observed in several species. Other related compounds have been isolated from different fungal sources (Figure 3).[14b, 14c, 16]



cis-Dehydrocurvularin

8-Methoxycurvularin

12-Chlorodehydrocurvularin



Citreofuran

8-Hydroxy-7-oxocurvularin

8,9-Epoxycurvularin

Figure 3 Other curvularin-related compounds

X-ray crystallographic studies show that dehydrocurvularin and curvularin adopt opposite helicity for the macrocyclic ring in the solid state.[17]

Pioneering biosynthetic studies on curvularin were done by Birch and co-workers in 1959.[18] A head-to-tail linkage of eight acetic acid units was proposed, based on the results of chemical degradation of curvularin derived from [^{14}C]acetic acid.

The biosynthesis of dehydrocurvularin by a strain of *Alternaria cinerariae* has been studied in some detail by our group, not only because of its potent phytotoxic activity, but also because different oxidation states exist along the polyketide chain. Earlier work on this compound established the origins of all constituent atoms and the pattern of bonds derived from acetate and aerobic oxygen (Figure 4).[19] It was found that the oxygen at C-10 can not be labeled by either acetate or $^{18}O_2$ gas. This suggests that it may be added by post-assembly oxidative processes.

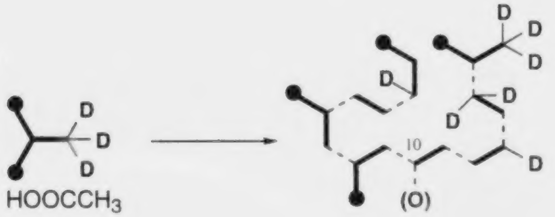
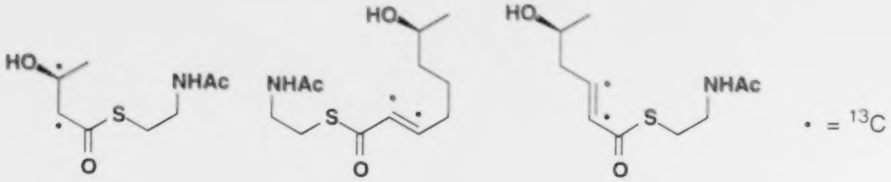


Figure 4 Pattern of bonds derived intact from acetate

It was also shown that ^{13}C-doubly labeled di- and tetra-ketides **4** and **5** (Figure 5), activated as their *N*-acetylcysteamine (NAC) thioesters, can be incorporated intact into dehydrocurvularin with the assistance of β-oxidation inhibitors.[10e, 10g] However, many attempts to achieve intact incorporation of the triketide **6** using a large variety of feeding protocols and β-oxidation inhibitors, with both mutant and wild type *A. cinerariae* strains were unsuccessful.[20]

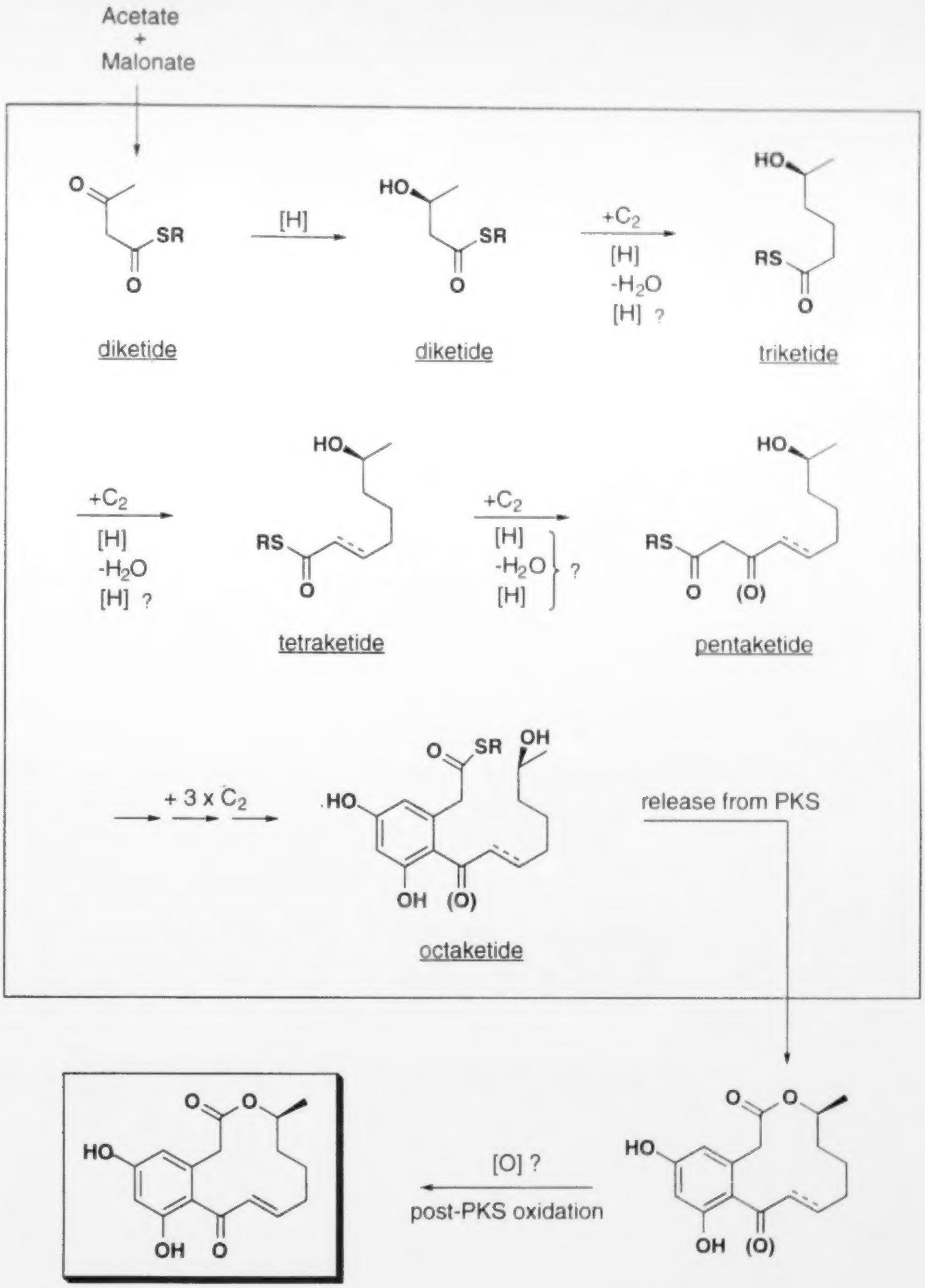


4 5 6

Figure 5 Previous targets for incorporation

Lai *et al.*[21] studied the biosynthesis of some curvularin-type metabolites in a hybrid strain ME 0005 derived from *Penicillium citreo-viride* B. IFO 6200 and 4692. ^{13}C NMR spectra of citreofuran, 7-oxocurvularin and 8-hydroxy-7-oxocurvularin show the same labeling patterns as those for **1** and **2**. These results suggest that all these curvularin-type metabolites are closely related biosynthetically.

Based on these experiments, an assembly mechanism outlined in Scheme 4 appears reasonable. Details for several steps remain unclear, such as the sequence of the late stage transformations. These could provide insight into the biogenic relationships among dehydrocurvularin, curvularin and 8-hydroxycurvularin. The focus of the present studies also included structural elucidation of the dehydrocurvularin PKS enzyme-bound intermediates. Thus, in order to gain more information about the assembly sequences, a number of putative advanced precursors were synthesized and examined for intact incorporation.


Acetate
+
Malonate
[H]
+C$_2$
[H]
-H$_2$O
[H] ?
diketide
diketide
triketide
tetraketide
pentaketide
+ 3 x C$_2$
octaketide
release from PKS
[O] ?
post-PKS oxidation

Scheme 4 Proposed assembly mechanism for dehydrocurvularin

RESULTS AND DISCUSSION

1. Synthesis and Incorporation of Diketide and Triketide

Since the hydroxyl-bearing diketide, precursor **4** can be incorporated intact into dehydrocurvularin (**1**), compounds with earlier oxidation states in the assembly pathway such as the keto form **7**, should also be intermediates. The reason for the failure to detect any incorporation of triketide precursor **6** remains unknown, but it is possible that there is a requirement for a *cis* double bond rather than the *trans* in the precursor. Attempts to make the *cis* isomer of **6** failed due to the complete isomerization of the *cis* double bond to the *trans* form during formation of the NAC thioester. Alternatively, it is possible that the triketide intermediate has to be fully saturated as it appears in dehydrocurvularin.

In order to test these possible assembly mechanisms, diketide **7** and triketide **8** precursors (Figure 6) were synthesized and administered to *A. cinerariae* to determine whether they can be incorporated intact. ^{13}C-labels are placed at α,β-positions since *in vivo* cleavage by β-oxidation would generate singly labeled acetates. These would be unlikely to recouple to a doubly labeled fragment due to dilution by unlabeled acetate from the fermentation medium.

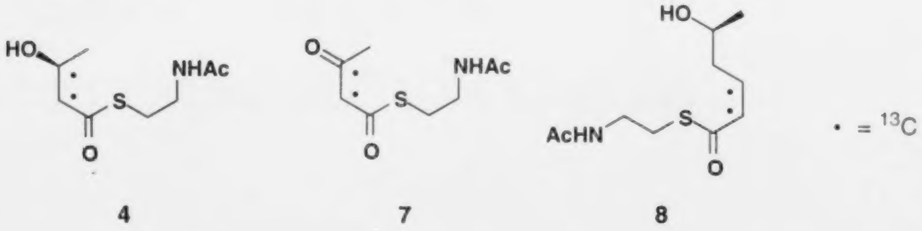
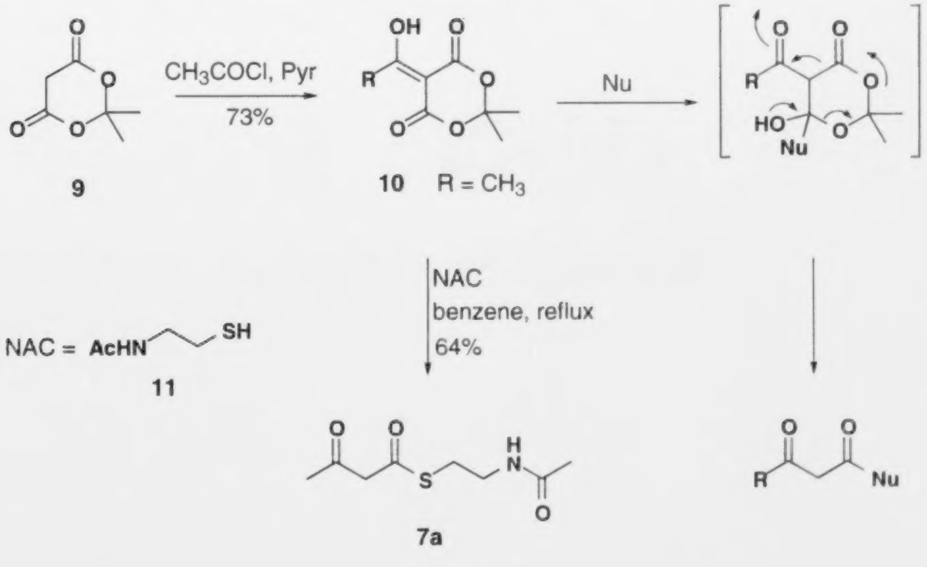


Figure 6 Diketide and triketide precursors

1.1 Synthesis of Diketide and Triketide Precursors

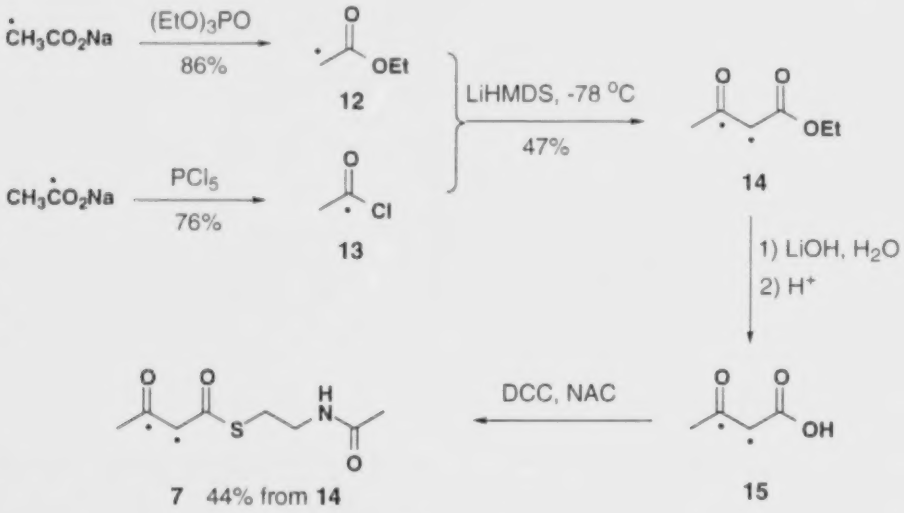
N-Acetylcysteamine (NAC) thioesters are generally prepared by coupling of the corresponding carboxylic acids with NAC in the presence of dicyclohexylcarbodiimide (DCC) with catalysis by dimethylaminopyridine (DMAP)[22]. Other methods include the use of acyl imidazolides[23] and acyl azides[24] prepared using carbonyl diimidazole (CDI) and diphenylphosphoryl azide (DPPA), respectively. Gilbert *et al.*[25] reported recently that the treatment of acylated Meldrum's acid with NAC gives good yields of NAC β-ketothioesters directly (Scheme 5), which avoids the potential decarboxylation of the corresponding free β-ketoacids.

Meldrum's acid **9** is readily acetylated with acetyl chloride, as described in the literature,[26] to give enol **10** in 73% yield (Scheme 5). Subsequent treatment with *N*-acetylcysteamine in refluxing benzene gives the expected β-ketothioester. However, the product co-elutes with the excess *N*-acetylcysteamine on silica gel, causing difficulty in purification. This problem can be overcome by using excess Meldrum's acid, to give NAC ester **7a** in 64% yield.



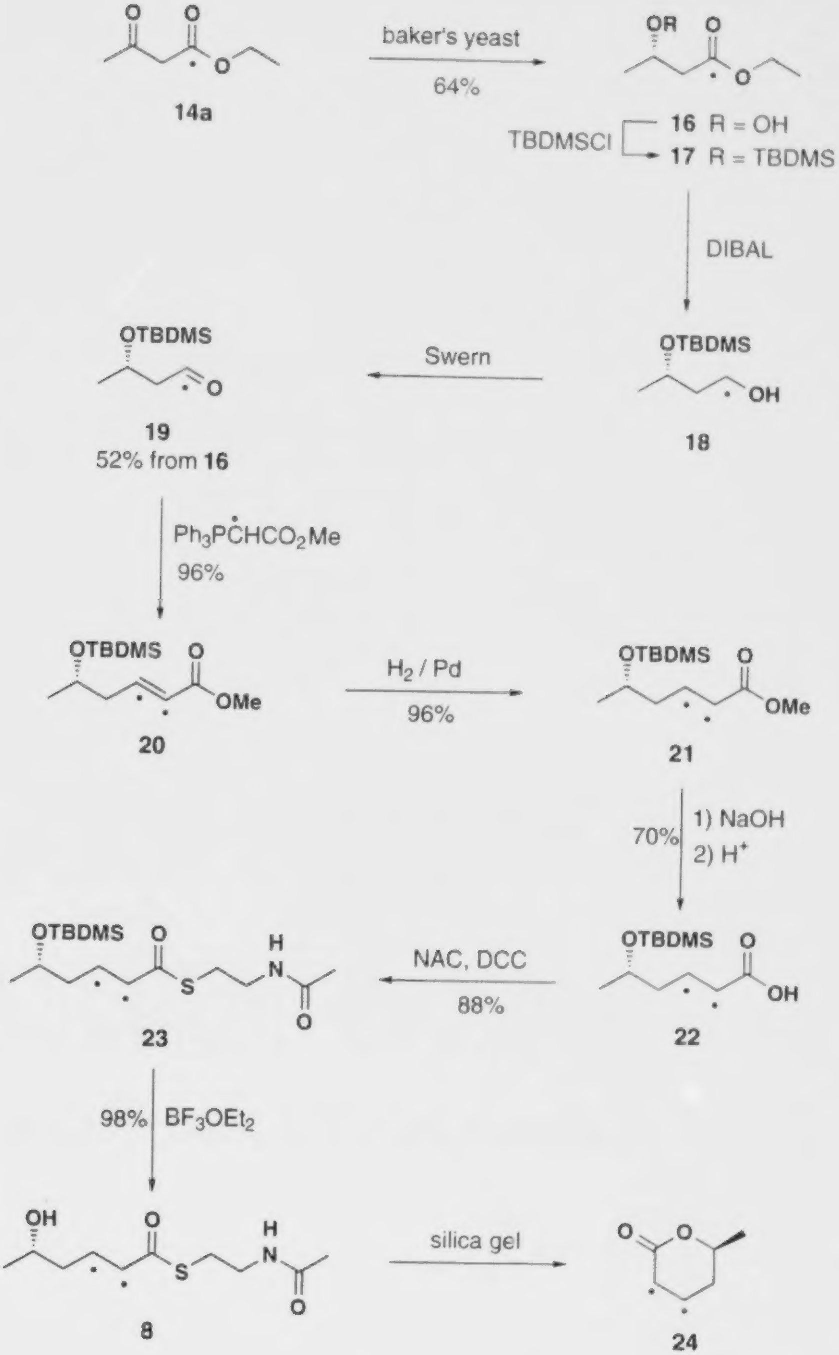
Scheme 5

To make α,β-^{13}C-doubly labeled precursor **7**, the conventional coupling method seems more practical, due to the cost of labeled materials. Thus ethyl [2,3-^{13}C]acetoacetate **14** is prepared from mono-labeled sodium acetates as shown in Scheme 6.[27] Careful hydrolysis of ethyl acetoacetate gives the corresponding acid **15**. Coupling with *N*-acetylcysteamine provides NAC ester **7** in 44% yield for two steps.



Scheme 6

The synthesis of the saturated triketide precursor **8** is easily achieved by the method developed by Dr. Zhe Li, a former graduate student in this laboratory.[27] Mono-^{13}C-labeled ethyl acetoacetate **14a** prepared in similar manner to **14** is reduced by baker's yeast[28] to give *S*-alcohol **16** (>90% ee) (Scheme 7). After protection of the hydroxyl group with TBDMS to yield **17**, the ester moiety is reduced to alcohol **18**. Swern oxidation converts the alcohol to aldehyde **19**, in 52% yield from **16**. Wittig reaction[29] extends the chain by another two carbons to give the doubly ^{13}C-labeled α,β-unsaturated ester **20** as the *E*-isomer, with no detectable amount of *Z*-isomer. Hydrogenation and saponification, followed by DCC coupling with *N*-acetylcysteamine provides NAC ester **23**.


14a
baker's yeast
64%
OR
16 R = OH
17 R = TBDMS
TBDMSCl
DIBAL
OTBDMS
OH
18
Swern
19
52% from 16
Ph$_3$PCHCO$_2$Me
96%
OMe
20
H$_2$ / Pd
96%
21
1) NaOH
2) H$^+$
70%
22
NAC, DCC
88%
23
98%
BF$_3$OEt$_2$
8
silica gel
24

Scheme 7

Treatment of **23** with tetrabutylammonium fluoride[30] (TBAF) in THF failed to remove the silyl group. However, reaction with boron trifluoride etherate[31] in cold dichloromethane affords the precursor **8** in 98% yield. As expected **8** cyclizes readily on silica gel to form known lactone[32] **24**. Therefore it has to be used for feeding experiments directly without further purification.

1.2 Incorporation of Diketide and Triketide Precursors

The wild type of *A. cinerariae* (ATCC 11784) was used for incorporation studies and was grown in potato dextrose broth (PDB) as reported in the literature.[27] During preliminary studies, careful separation of the extracts afforded a new metabolite. Its high resolution EI-MS spectrum is very similar to that of dehydrocurvularin (**1**), except that it shows an additional small peak at *m/z* 308 corresponding to $C_{16}H_{20}O_6$, which was confirmed as the molecular ion by CI-MS (NH_3). ^1H and ^{13}C NMR spectrometry show that it exists as a mixture of two isomers (1:2.6). A unique multiplet at 4.11 ppm in the ^1H NMR spectrum indicates the existence of a secondary alcohol. This compound has therefore been identified as 8-hydroxycurvularin (**3**), previously isolated from *Alternaria tomato*[15c] and *Penicillium* sp. 511.[14c] Production curves for **1** and **3** are shown in Figure 7. Under typical conditions the fungus can produce 200 mg of **1** and 50 mg of **3** per litre of broth.

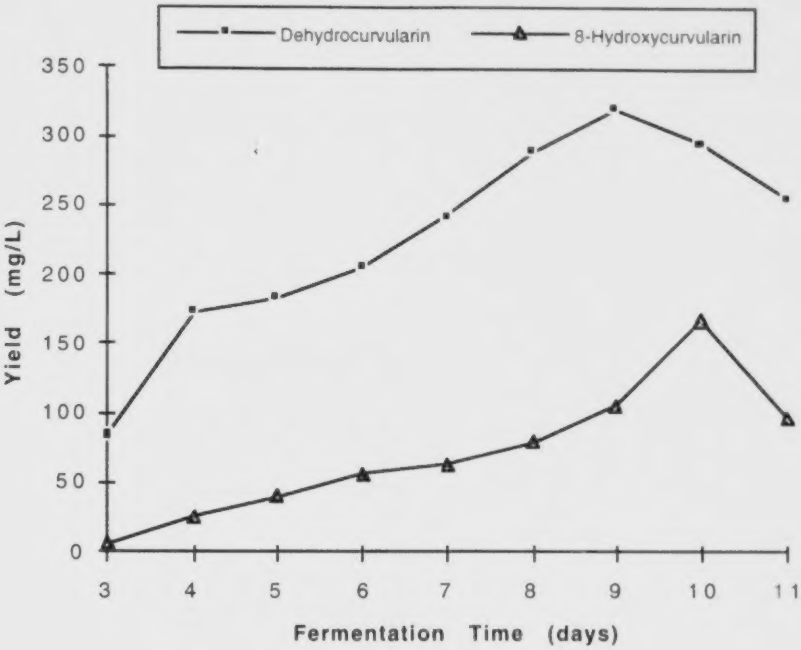
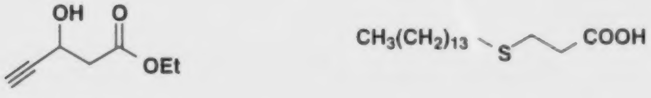


Figure 7 Production curves for dehydrocurvularin and 8-hydroxycurvularin

It has been reported that high glucose media can suppress β-oxidation.[12] Therefore the feeding experiment was done in replacement media and in the presence of a β-oxidation inhibitor (Figure 8).



25 **26**

Figure 8 β-Oxidation inhibitors

A solution of diketide **7** (37 mg) and ethyl 3-hydroxypentynoate[27] (**25**) (35 mg) in 98% ethanol (2.5 mL) was administered in six aliquots to the high glucose fermentation culture of *A. cinerariae* (125 mL). The analysis of the resulting dehydrocurvularin sample by ^1H-decoupled ^{13}C NMR spectrometry shows coupled signals at 72.82 and 34.73 ppm with a coupling constant of 38.8 Hz, corresponding to C-4 and C-5 respectively (Figure 9). Diketide **7** showed 0.24% intact incorporation based on the integration ratio (22%) of the coupled signals to the natural abundance signal.

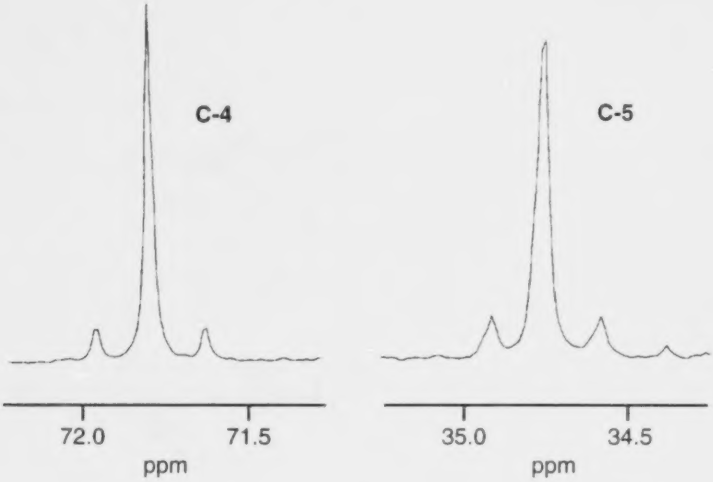


Figure 9 Expansions of ^{1}H-decoupled ^{13}C NMR spectra of **1** after incorporation of **7**

However, addition of triketide **8** to *A. cinerariae* in the presence of 3-(tetradecylthio)-propanoic acid[33] (**26**) as the β-oxidation inhibitor did not show any incorporation. This suggests that the PKS machinery may permit loading of external precursors only at certain chain lengths or oxidation states which allow effective competition with the endogenously generated intermediates that are already covalently attached to the PKS. Other possible reasons for the lack of successful intact incorporation may be inability of the substrate to cross the cell membrane or rapid breakdown of the exogenously added precursor by cellular degradation pathways such as thioester hydrolysis or β-oxidation. It presently remains unknown why triketide incorporation fails whereas di- and tetraketides can be utilized.

2. Synthesis and Incorporation of Tetraketides

The coexistence of dehydrocurvularin (**1**) and 8-hydroxycurvularin (**3**) in *A. cinerariae*, as well as the co-occurrence of **1** with curvularin (**2**) in other species, suggests that the oxidation states of C-8 and C-9 in the intermediates along the biosynthetic pathway are not predictable. Although intact incorporation of unsaturated tetraketide **5** may initially suggest that its double bond and carbonyl oxygen are utilized directly, this may not be the case since the double bond may be fully reduced prior to the next chain elongation step, and subsequently reoxidized in the post-PKS processes. Therefore, we decided to examine the NAC ester of the fully saturated tetraketide **27** (Figure 10).

A second aspect of interest was the scope of acceptable "carrier" moieties to assist incorporation. NAC was developed by Lynen as a truncation of the acyl carrier protein in his studies of fatty acid synthesis.[34] NAC thioesters are so far the best derivatives of carboxylic acids that have been developed for incorporation of advanced precursors. As previously mentioned, one of the reasons for failure of intact incorporation may be inefficient transport of the precursor across the cell membrane. Therefore, it seemed desirable to develop a NAC derivative with an additional functionality for attachment of molecules like sugars, which would undergo active transport.[35] For this purpose, compound **28** could be a potential mimic of the 4'-phosphopantetheine moiety of acyl carrier protein. Since the unsaturated tetraketide **5** shows intact incorporation into dehydrocurvularin, it provides a suitable system to check whether thioesters of the modified NAC **28** (e.g. compound **29**) can be accepted or not.

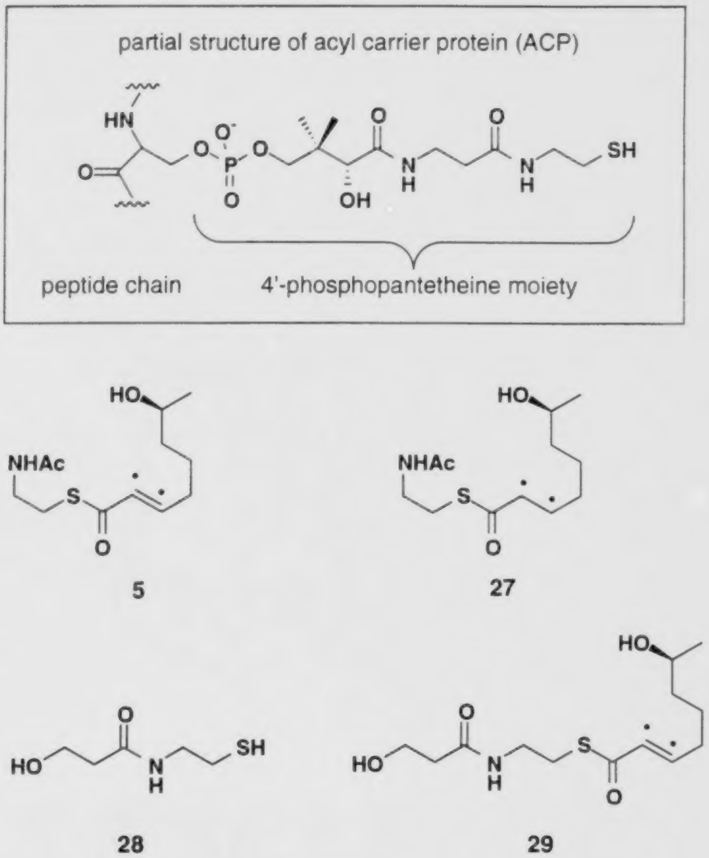
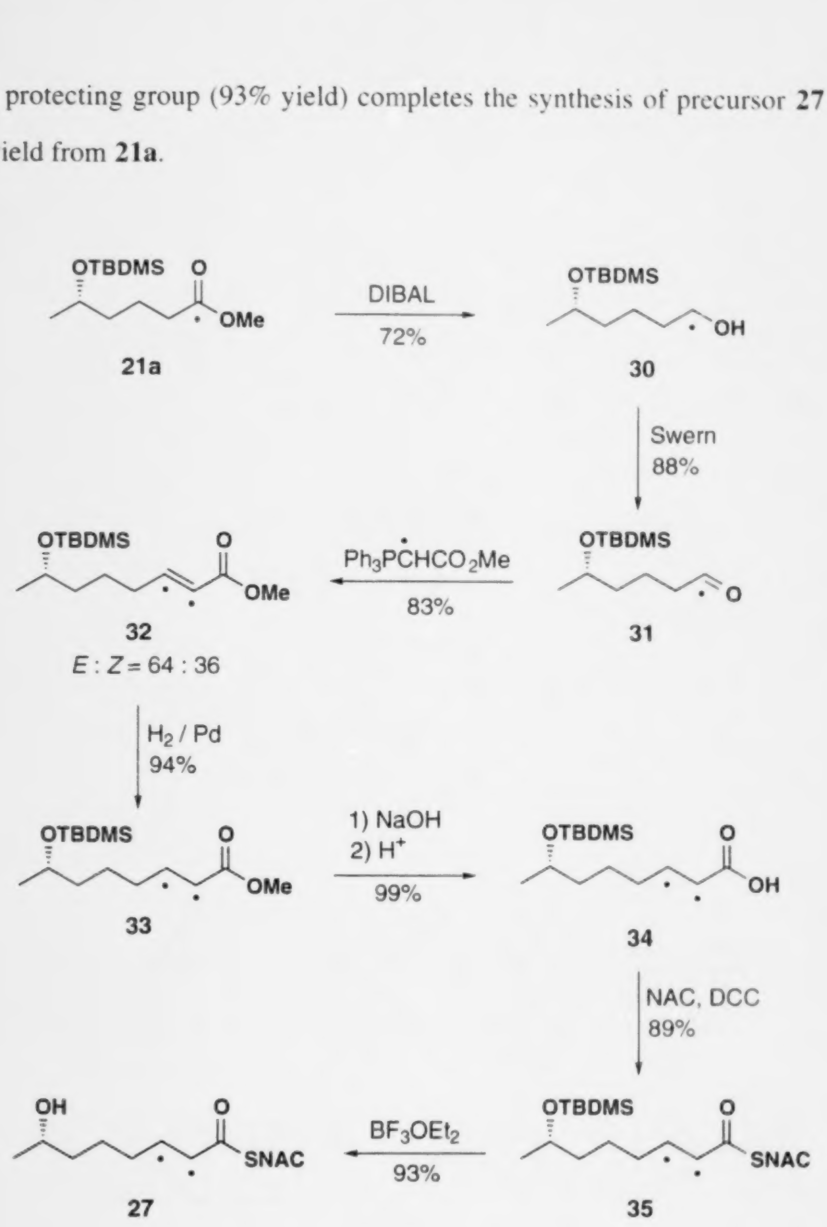

partial structure of acyl carrier protein (ACP)
HN
O
O
P
O
O
O
OH
O
N
H
O
N
H
SH
peptide chain
4'-phosphopantetheine moiety
HO
NHAc
S
O
5
HO
NHAc
S
O
27
O
HO
N
H
SH
28
HO
O
HO
N
H
S
O
29

Figure 10

2.1 Synthesis of the Saturated Tetraketide

The saturated tetraketide precursor **27** was synthesized as shown in Scheme 8, adopting the earlier strategy described for the synthesis of **8**. A key feature is that ^{13}C-labels are incorporated into the molecules at a late stage of the synthesis in order to achieve high efficiency of isotope utilization.

Mono-labeled compound **21a** is available in a similar manner to the doubly-labeled compound **21** (Scheme 7). This could then be extended by two carbons to give **32** as mixture of two isomers, E:Z = 64:36. Hydrogenation of the mixture gives the saturated ester **33**, which is hydrolyzed and coupled with NAC to form NAC ester **35**. Removal of the silyl protecting group (93% yield) completes the synthesis of precursor **27** in 41% overall yield from **21a**.

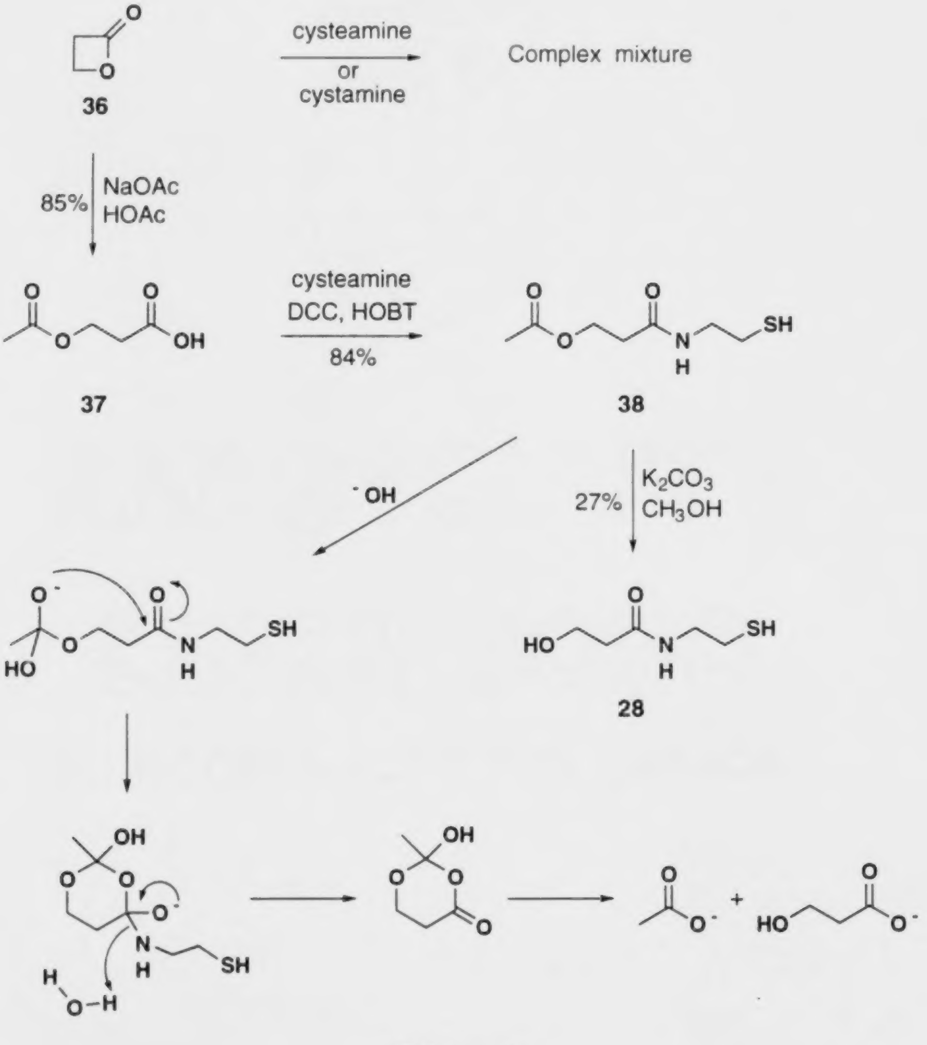

OTBDMS
O
OMe
21a
DIBAL
72%
OH
30
Swern
88%
31
$Ph_3\dot{P}CHCO_2Me$
83%
32
E : Z = 64 : 36
H_2 / Pd
94%
33
1) NaOH
2) H^+
99%
34
NAC, DCC
89%
35
BF_3OEt_2
93%
OH
SNAC
27

Scheme 8

2.2 Modification of NAC

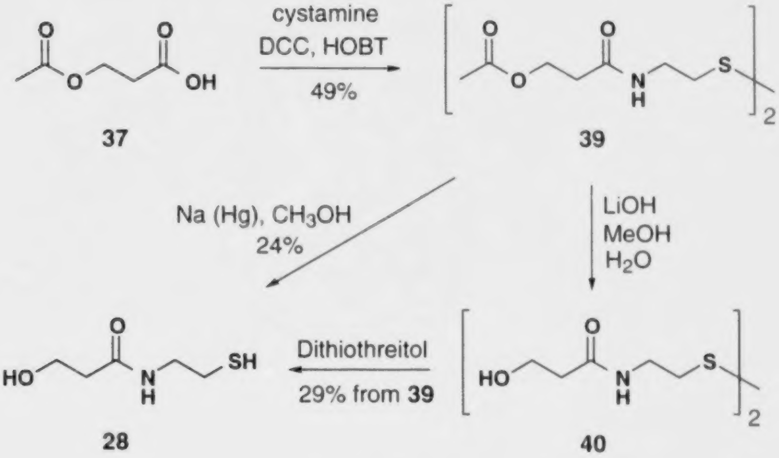
The synthesis of the modified NAC derivative **28** starts from the commercially available β-propiolactone **36** (Scheme 9). Direct nucleophilic attack onto **36** with cysteamine or its disulfide cystamine, always gives complicated products because the β-lactone can undergo both *O*-acyl and *O*-alkyl cleavages and also polymerizes easily.[36]

However, treatment of **36** with sodium acetate in the presence of acetic acid generates compound **37** in good yield. Selective coupling with the amino end of cysteamine forms the amide **38**. The hydrolysis of the acetate moiety of **38** is unexpectedly problematic. A number of saponification procedures were examined, but all give water-soluble fragmentation products. Mild conditions, such as potassium carbonate in dry methanol produce **28** in low yield.[37] The decomposition of **38** under basic conditions may occur by acetate-assisted amide hydrolysis, as shown in Scheme 9.


36
cysteamine
or
cystamine
Complex mixture
85%
NaOAc
HOAc
37
cysteamine
DCC, HOBT
84%
38
⁻OH
27%
K2CO3
CH3OH
28
OH
SH
HO

Scheme 9

In an alternative approach to **28**, acid **37** could be coupled with cystamine, which serves as a protected form of cysteamine, to afford **39** in 49% yield (Scheme 10). Hydrolysis of **39** gives the diol **40**. Reduction of **40** with dithiothreitol[38] is very sluggish, even with a three fold excess of reagent. Compound **28** is obtained in 29% yield (for two steps), with 36% of **40** recovered. Sodium amalgam[39] reduction of **39** produces **28** directly in low yield.

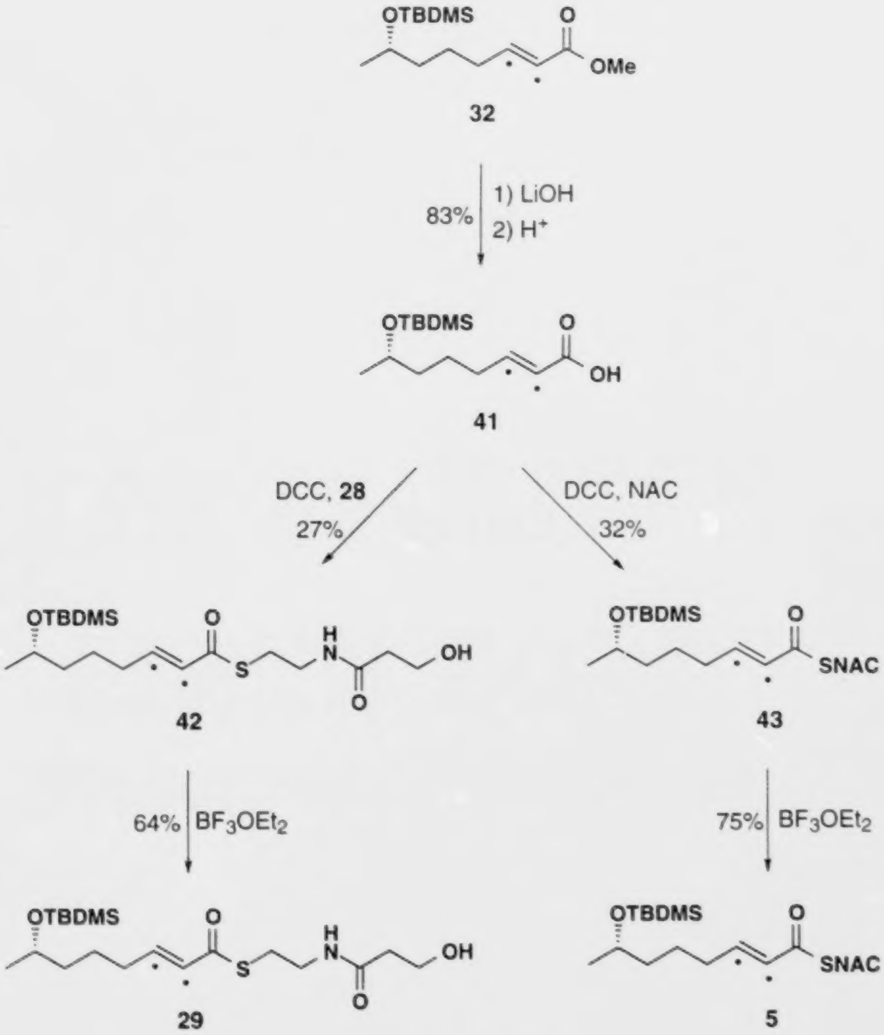


Scheme 10

The doubly ^{13}C-labeled acid **41** is available by LiOH saponification of methyl ester **32** in 83% yield (Scheme 11). Coupling of the modified NAC derivative **28** with **41** by the standard procedure forms the desired product **42**, *via* reaction with the more nucleophilic thiol end. Removal of the silyl group with boron trifluoride etherate completes the synthesis of precursor **29**. For comparison purposes compound **5** was prepared as previously described by Dr. Zhe Li in our group.[27]

2.3 Incorporation of Tetraketides

Feeding of **27**, **29** and **5** to cultures of wild type *A. cinerariae* employed identical conditions to those used for the triketide **8**, with 3-(tetradecylthio)propanoic acid (**26**) as the β-oxidation inhibitor. The saturated NAC ester **27** and the unsaturated tetraketide **29** with the modified NAC derivative did not show any detectable intact incorporation, but unsaturated NAC ester **5** showed substantial intact incorporation into **1** under identical feeding conditions. Thus, the ^{1}H-decoupled ^{13}C NMR spectrum of **1** derived from **5** shows two sets of coupled signals for C-8 and C-9 at 149.6 and 132.5 ppm as expected (Figure 11).


OTBDMS
O
OMe
32
83%
1) LiOH
2) H⁺
OTBDMS
O
OH
41
DCC, 28
27%
DCC, NAC
32%
OTBDMS
O
S
H
N
O
OH
42
OTBDMS
O
SNAC
43
64%
BF3OEt2
75%
BF3OEt2
OTBDMS
O
S
H
N
O
OH
29
OTBDMS
O
SNAC
5

Scheme 11

In addition intact incorporation of **5** into 8-hydroxycurvularin (**3**) occurs. In the ^{1}H-decoupled ^{13}C NMR spectrum of **3** (Figure 12), two sets of coupled signals appear for the resonances of C-8 and C-9, at 67.07 and 54.50 ppm with a coupling constant of 36.1 Hz for the major isomer, and at 67.58 and 53.82 ppm with a coupling constant of 36.3 Hz for the minor isomer.

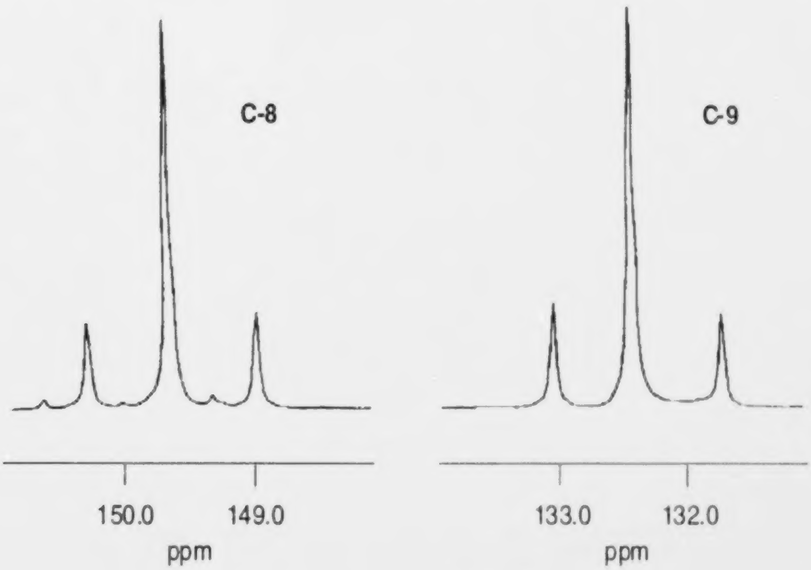


Figure 11 Expansions of ^{1}H-decoupled ^{13}C NMR spectra of **1** after incorporation of **5**

These incorporation results indicate that the double bond is essential for incorporation at the tetraketide stage. They also suggest that the modified NAC is not acceptable to the polyketide synthase machinery.

In order to check the possibility that the saturated tetraketide **27** could be utilized to produce curvularin (**2**), a sample of **2** was mixed with the extracts of fungal cultures obtained after fermentation with **27**, and the curvularin fraction was re-isolated and purified. The mass spectrum of the curvularin sample obtained in this manner is identical to that of natural curvularin. This result indicates that there is no detectable incorporation of **27** into curvularin (**2**) by *A. cinerariae*.

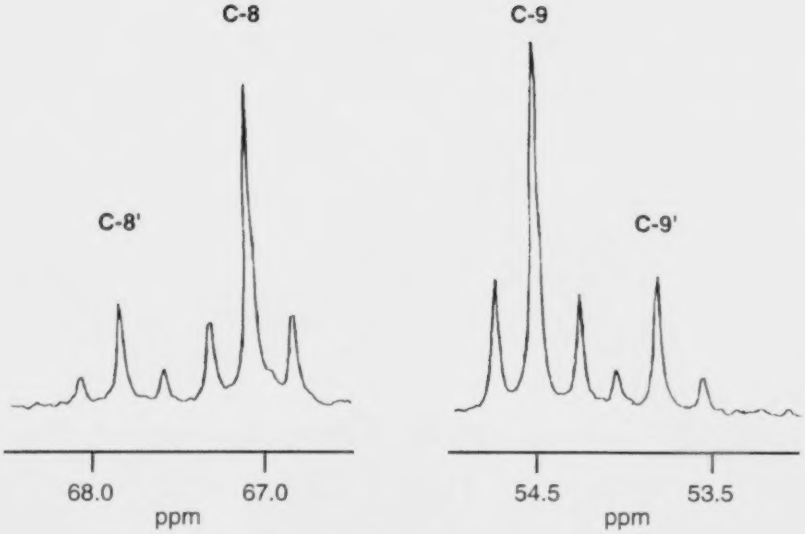


Figure 12 Expansions of ^{1}H-decoupled ^{13}C NMR spectra of **3** after incorporation of **5**

Dr. Yuko Yoshizawa, a former post-doctoral fellow in this group, has shown that wild type *A. cinerariae* produces curvularin (**2**) in addition to dehydrocurvularin (**1**) in stationary media. This provides a better opportunity to examine the incorporation of **27** into curvularin. Stationary culture fermentation proceeded in a 2.8 L Fernbach flask at 28 °C in the absence of light. The culture was harvested 20 days after inoculation to provide approximately 7.5 mg of curvularin (**2**) per litre broth, which is about 10% of the production of dehydrocurvularin (**1**). Submerged fermentation at low rotation speed (70 rpm) gives the same metabolite profile, but increases the yield of curvularin to 10 mg per litre of broth. Separate feeding of **27** and **5** was done under low speed submerged conditions with high glucose media replacement. The production of **1** and **2** was remarkably depressed by the lower shaking speed. Curvularin (**2**) samples obtained this way were not sufficient to proceed with ^{13}C NMR spectrometric analysis. The dehydrocurvularin (**1**) samples produced were examined with ^{13}C NMR spectrometry. Surprisingly, they did not show any incorporation either for saturated NAC ester **27** or for unsaturated NAC ester **5**. Since a substantial incorporation for **5** occurs at normal rotation speed (160 rpm), this suggests that at low speed or stationary culture conditions, the precursors can not enter the cells efficiently to compete with the enzyme-bound intermediates.

3. Post-PKS Transformation

It has been widely accepted that after assembly of the polyketide skeleton by the polyketide synthase, the initial PKS product can be released from the enzyme and further modified by a variety of post-PKS processes, such as oxidation, alkylation and glycosylation to give the final metabolites. As mentioned in the introduction, the origin of the oxygen atom at C-10 remains unclear since it is not labeled by either [^{18}O]acetate or oxygen-18 gas. Knowledge of the oxidation state at this position is crucial to the design of putative pentaketide intermediates.

3.1. Blocking the Post-Assembly Processes

Cytochrome P-450 dependent oxidation[40] often occurs in the biosynthesis of secondary metabolites, such as steroids,[41] terpenoids[42] and flavonoids.[43] This type of oxidation usually takes place in the late stages of biosynthesis and can be important for biological activity. Various inhibitors for P-450 enzymes have been developed as clinical drugs and plant growth regulators,[44] e.g. acymidol (**44**) and S-3307D (**45**) (Figure 13). They possess lipophilic and heteroaromatic moieties which reversibly bind the lipophilic domain and haem iron, thereby, blocking binding of the substrate and molecular oxygen.

Based on this rather simple mechanism, P-450 inhibitors have been successfully used to suppress the oxidative modification of several secondary metabolites, resulting in accumulation of less oxidized products. Acymidol (**44**) and metyropone (**46**) were effectively used for the biosynthetic studies of betaenone B[45] and chaetoglobosin,[46] isolated from fungi *Phoma betae* and *Chaetomium subaffine*, respectively. New less oxidized metabolites were isolated and identified as plausible precursors in the biosynthetic pathways.

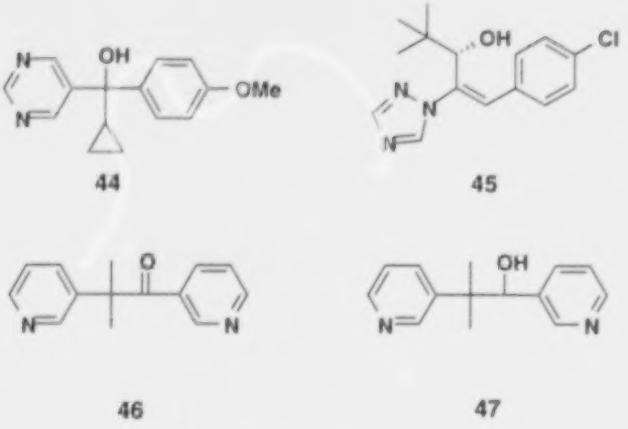


Figure 13

This approach was applied to our system in an effort to determine the oxidation state at C-10 of the initial PKS product in the path to dehydrocurvularin (**1**). Acymidol (**44**) was synthesized according to the literature method[47] by nucleophilic addition of the pyrimidine moiety to the corresponding ketone, and then administered to the fungus *A. cinerariae*. Commencing two days after inoculation with *A. cinerariae*, equal portions of acymidol (0.05 M in 70% ethanol) were added for five days to the cultures (final concentration 0.10-10.0 mM). It was found that a high concentration of acymidol (final concentration >2.0 mM) killed the fungus. At lower concentrations of the inhibitor, the growth of the fungus was suppressed to some extent, but sufficient quantities of metabolites were produced for analysis. Examination by HPLC and comparison with a blank in which no inhibitor was employed, shows no difference except for the presence of a peak due to acymidol.

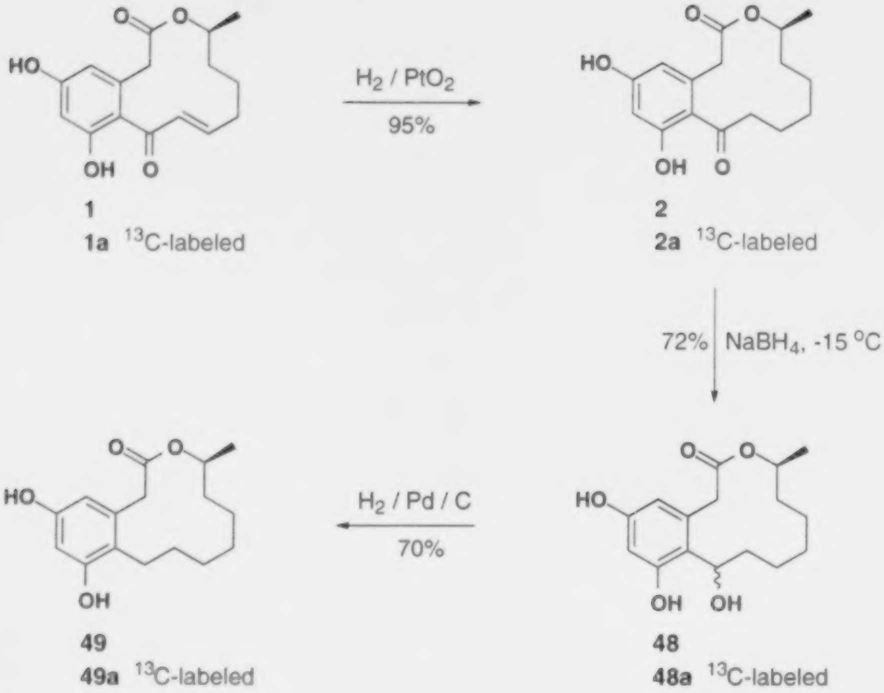
Similarly, 0.1 mM of metyropone in 70% ethanol was added to the fungus one day after the inoculation in equal portions for five days (final concentration 0.5-5.0 mM).

The results show that metyropone does not significantly affect the metabolite types and amounts, and that the inhibitor itself is reduced by the fungus to the corresponding alcohol **47** in all cases.

3.2. Feeding of Advanced Precursors

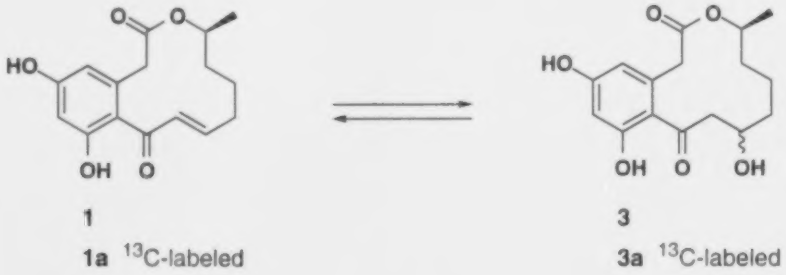
An alternative way to determine the initial oxidation state of C-10 is to start from acetate-labeled dehydrocurvularin. Using chemical transformations, all the putative derivatives with different oxidation states at that position can be synthesized. The labeled derivatives can then be fed back to the fungus to see if these compounds are transformed to dehydrocurvularin by the post-PKS enzymes.

This approach was pursued, and the chemical transformations employed are outlined in Scheme 12. The synthesis was first developed using unlabeled dehydrocurvularin (**1**). Hydrogenation of **1** in the presence of PtO_2 converts it to curvularin (**2**) in 95% yield.[19] Reduction with sodium borohydride at -15 °C gives alcohol **48** as a mixture of two isomers (ca. 1:1 ratio). Removal of the benzylic hydroxyl of **48** by hydrogenolysis affords 10-dihydrocurvularin (**49**). Labeled **2a**, **48a** and **49a** are available in the same way from acetate-labeled **1a**, which is isolated from the culture of *A. cinerariae* fed with doubly ^{13}C-labeled sodium acetate. Additions of the labeled **2a**, **48a** or **49a** in either the presence or absence of β-oxidation inhibitor **26** did not result in any incorporation of these precursors into dehydrocurvularin (**1**).


H2 / PtO2
95%
1
1a 13C-labeled
2
2a 13C-labeled
72%
NaBH4, -15 °C
H2 / Pd / C
70%
49
49a 13C-labeled
48
48a 13C-labeled

Scheme 12

During the incorporation experiments it became apparent that dehydrocurvularin (**1**) and 8-hydroxycurvularin (**3**) are in equilibrium in the fungal culture (Scheme 13). Thus, addition of labeled dehydrocurvularin (**1a**) to the fermentation culture of *A. cinerariae* generates 8-hydroxycurvularin (**3a**) which shows the same labeling pattern and approximately the same enrichment of carbon-13. Similarly, addition of labeled **3a** to the cultures gives labeled **1a**.

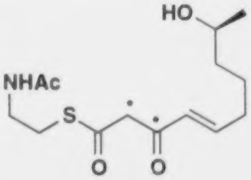

1
1a 13C-labeled
3
3a 13C-labeled

Scheme 13

To understand the relationship between these two metabolites and to determine whether the interconversion is enzyme-mediated, a series of experiments were performed. The fungal culture is slightly acidic (ca. pH 5.5-6.0) throughout the fermentation. However, incubation of dehydrocurvularin (**1**) in pH 5 or pH 6 phosphate buffers at 28 °C shows no detectable formation of 8-hydroxycurvularin (**3**), thereby excluding simple acid-catalyzed hydration or dehydration. As a control, the fermentation culture of *A. cinerariae* was autoclaved at 121 °C for 20 min to kill the fungus, and acetate-labeled dehydrocurvularin (**1a**) was added. Surprisingly, the autoclaved mixture can still perform the transformation. Finally, acetate-labeled dehydrocurvularin (**1a**) was added to sterile potato dextrose media without fungus, and incubated under the same conditions. It was found that the media alone also accomplishes this transformation. This inter-conversion can be interpreted as a Michael addition and elimination of water catalyzed by some component(s) in the potato dextrose broth.

4. Attempts to Synthesize the Pentaketide

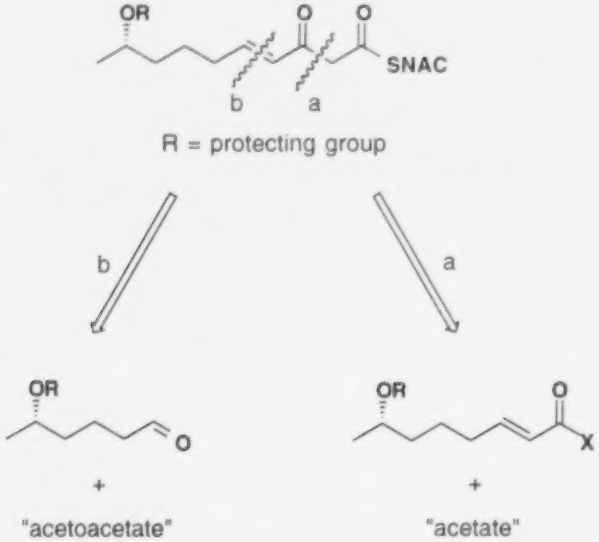
The studies on the tetraketides and late stage transformations show that the double bond in the tetraketide is essential for intact incorporation, and P-450 inhibitors can not cause accumulation of any metabolite less oxidized than dehydrocurvularin (**1**). Based on these results, a putative pentaketide precursor **50** was designed (Figure 14).



50

Figure 14 Putative pentaketide precursor

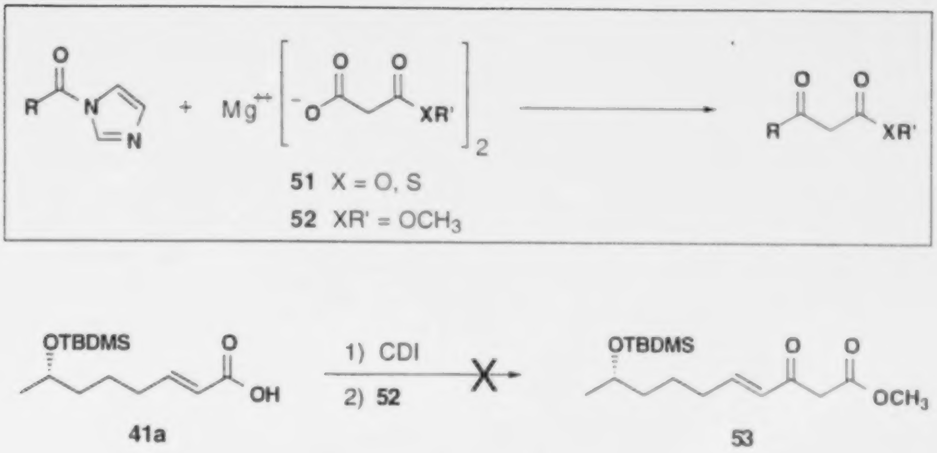
This molecule can be retrosynthetically disconnected in at least two ways (Scheme 14). The first approach is to cut between the α and β positions to give an eight carbon unit and an "acetate" equivalent (pathway a). The second one is to disconnect at the double bond to give a six carbon unit and an "acetoacetate" equivalent (pathway b). These approaches were first explored with unlabeled compounds.



Scheme 14

4.1 Synthetic Route "a"

Bram and Vilkas[48a] reported an elegant method for the synthesis of β-ketoesters or β-ketothioesters, which was later elaborated and developed by Brooks *et al.*[48b] Reaction of an acyl imidazolide with the magnesium salt of malonic acid half ester **51** results in an efficient *C*-acylation under virtually neutral conditions (Scheme 15). This procedure was tried with the unsaturated acid **41a**. Treatment with *N,N'*-carbonyldiimidazole (CDI) converts the acid **41a** to its imidazolide, but subsequent reaction with **52** gives complex mixtures, possibly because the double bond deactivates the carbonyl group and allows Michael addition reactions.



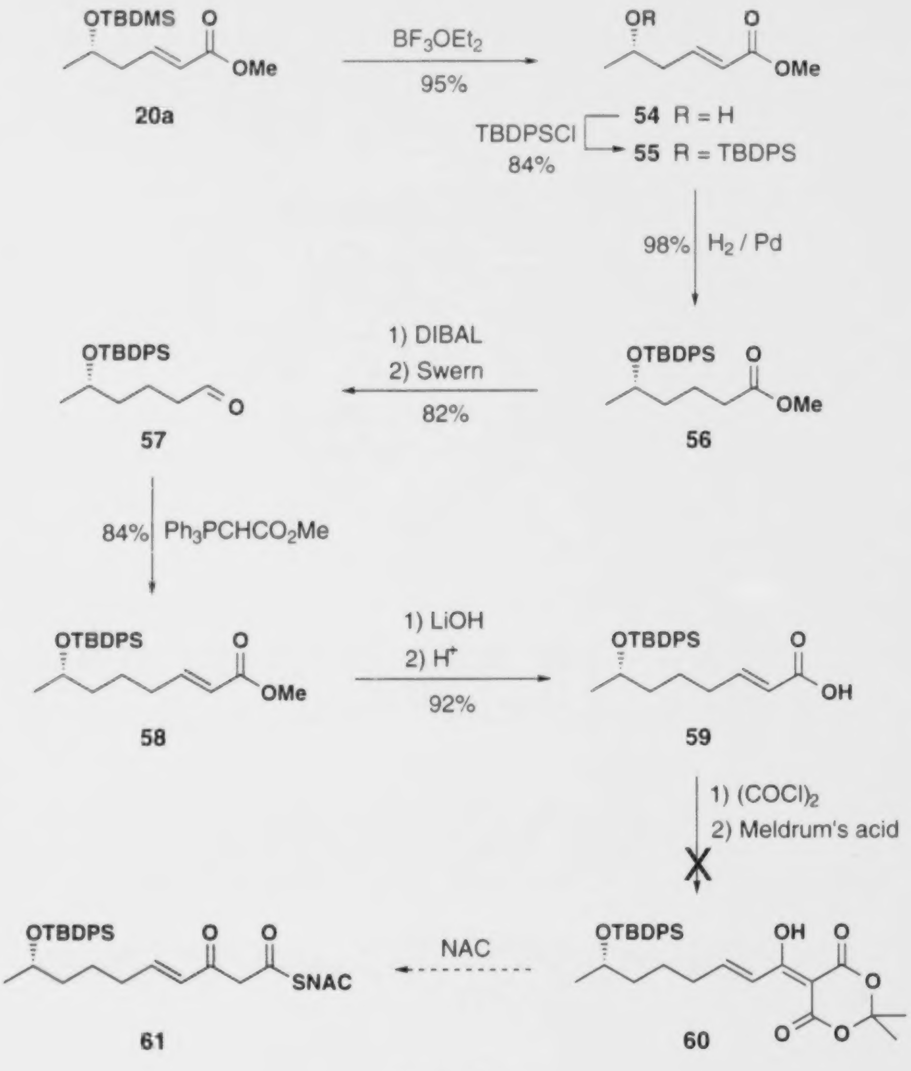
Scheme 15

An alternative procedure[49] involving α,β-unsaturated acids and a magnesium complex of ethyl hydrogen malonate would require conversion of **41a** to its acid chloride. In order to check the the stability of the TBDMS protecting group to these conditions, acid **41a** was treated with oxalyl chloride in the presence of a catalytic amount of DMF in dichloromethane, and the mixture was then quenched with methanol. As expected, this resulted in loss of the silyl group and formation of the methyl ester.

Condensation with Meldrum's acid[50] is an alternative means to make β-ketothioesters, as discussed in section 1.1 (Scheme 5). The main advantage of this method is that after the acylation the product can be directly converted to the β-ketothioester *via* nucleophilic attack of NAC. However, thus far, acid chlorides are the only effective acylating reagents. Model studies with the imidazolide of oct-2-enoic acid show no reaction with Meldrum's acid.

The *t*-butyldiphenylsilyl group (TBDPS) has been reported to be more acid resistant than the *t*-butyldimethylsilyl group (TBDMS);[51] the TBDPS ether is stable to 80% acetic acid and HBr/AcOH at 12 °C. Hence, TBDPS protected acid **59** was prepared using a procedure analogous to that for synthesis of **41a** (Scheme 16). Unfortunately, the reaction gave complex mixtures when **59** was treated with oxalyl chloride followed by

Meldrum's acid. However, the acid chloride generated by treating **59** with oxalyl chloride can be trapped by methanol to form the methyl ester **58**, showing that the TBDPS group is stable under the reaction conditions.



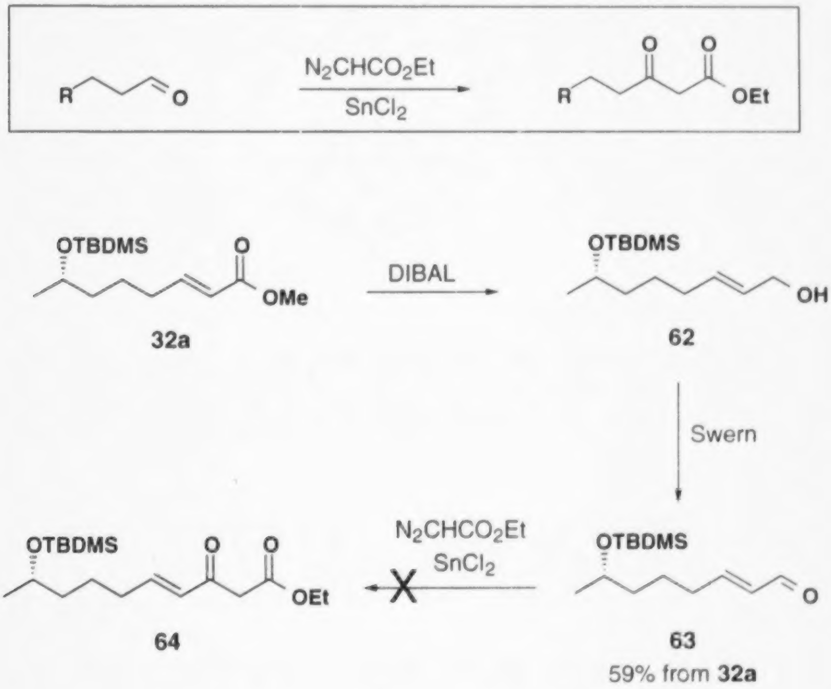
OTBDMS
O
OMe
20a
BF3OEt2
95%
OR
O
OMe
TBDPSCl
84%
54 R = H
55 R = TBDPS
98%
H2 / Pd
OTBDPS
O
OMe
56
1) DIBAL
2) Swern
82%
OTBDPS
O
57
84%
Ph3PCHCO2Me
OTBDPS
O
OMe
58
1) LiOH
2) H+
92%
OTBDPS
O
OH
59
1) (COCl)2
2) Meldrum's acid
OTBDPS
OH
O
O
O
O
60
NAC
OTBDPS
O
O
SNAC
61

Scheme 16

It has also been reported that aldehydes condense with ethyl diazoacetate in the presence of a Lewis acid to provide β-ketoesters;[52] the best results were obtained with

BF_3, $SnCl_2$ and $GeCl_2$ (Scheme 17). Reduction of the α,β-unsaturated ester **32a** with diisobutylaluminum hydride (DIBAL) gives alcohol **62**, with some contamination by the saturated analogue. Swern oxidation produces aldehyde **63**. Unfortunately, treatment of **63** with ethyl diazoacetate gives no reaction. The original study shows that the yields and relative rates are lower with aromatic aldehydes. Apparently the conjugated aldehyde **63** is simply too unreactive for this process.

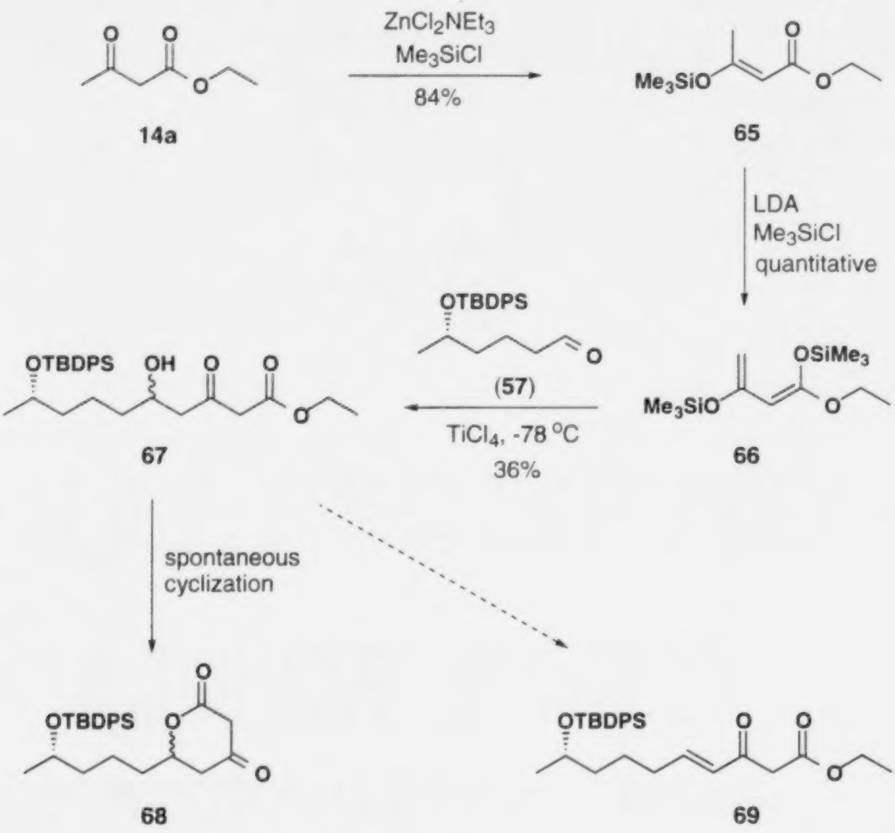
The difficulties inherent in applying known methodologies to extend the chain by two carbons led us to examine four carbon extensions ("strategy b").


R
O
N2CHCO2Et
SnCl2
R
O
O
OEt
OTBDMS
O
OMe
32a
DIBAL
OTBDMS
OH
62
Swern
OTBDMS
O
63
59% from 32a
N2CHCO2Et
SnCl2
OTBDMS
O
O
OEt
64

Scheme 17

4.2 Synthetic Route "b"

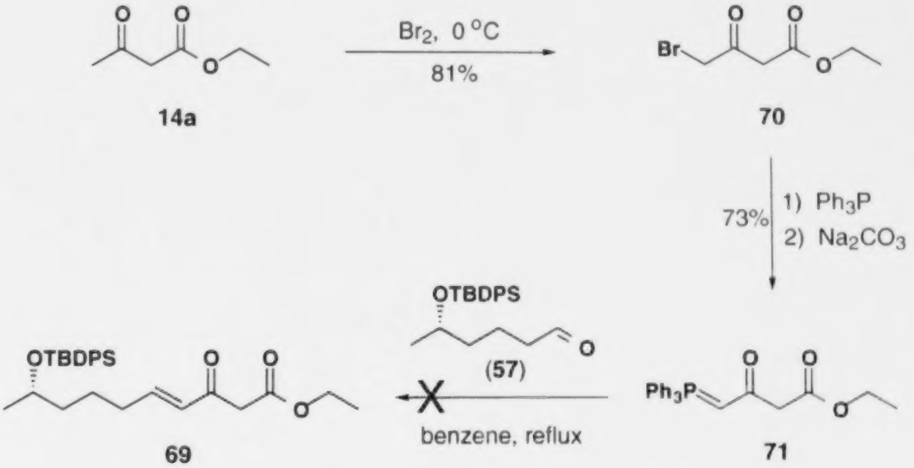
One of the "acetoacetate" equivalents is 1,3-bis(trimethylsilyl)-1-ethoxybuta-1,3-diene (**66**), which condenses with aldehydes in the presence of titanium tetrachloride to give aldol-type products.[53] The bis-trimethylsilyl compound **66** is readily prepared by stepwise silylation of ethyl acetoacetate as described in the literature[54] (Scheme 18). Reaction of aldehyde **57** with **66** in the presence of titanium tetrachloride produces the expected product **67** in 36% yield as a mixture of two isomers (ca. 1:1 ratio). However, the facile spontaneous cyclization to **68** prevents the conversion of **67** to the unsaturated compound **69**.



14a
ZnCl2NEt3
Me3SiCl
84%
Me3SiO
65
LDA
Me3SiCl
quantitative
OTBDPS
O
(57)
TiCl4, -78 °C
36%
OSiMe3
Me3SiO
66
OTBDPS OH O O
67
spontaneous cyclization
OTBDPS
68
OTBDPS
69

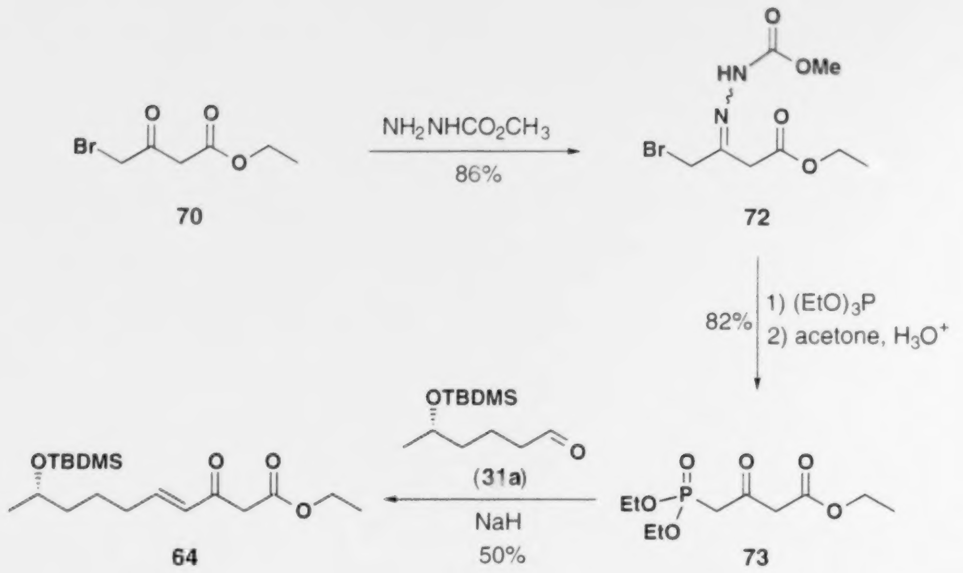
Scheme 18

Stabilized Wittig reagent **71**, which can be readily prepared from ethyl acetoacetate, has been widely used in organic synthesis as an "acetoacetate" equivalent (Scheme 19).[55] Bromination of ethyl acetoacetate in dichloromethane at 0 °C gives the thermodynamically more stable ω-brominated product **70**.[56] Reaction with triphenylphosphine forms the Wittig salt, which gives the ylide **71** upon treatment with sodium carbonate. The reaction between **71** and the aldehyde **57** is so sluggish that no condensation occurs even in refluxing benzene. Therefore, a more reactive version of this reagent was needed.



Scheme 19

The phosphonate reagent **73** is easily prepared by literature procedure (Scheme 20).[57] The carbonyl group of bromide **70** is protected with methyl hydrazinocarboxylate to form the corresponding hydrazone **72**. Arbuzov reaction of **72** in refluxing toluene, followed by deprotection in acidic acetone-water generates the reagent **73** in good yield. Reaction of **73** with aldehyde **31a** in the presence of sodium hydride gives the expected δ.,γ-unsaturated β-ketoester **64** in 50% yield.[58]

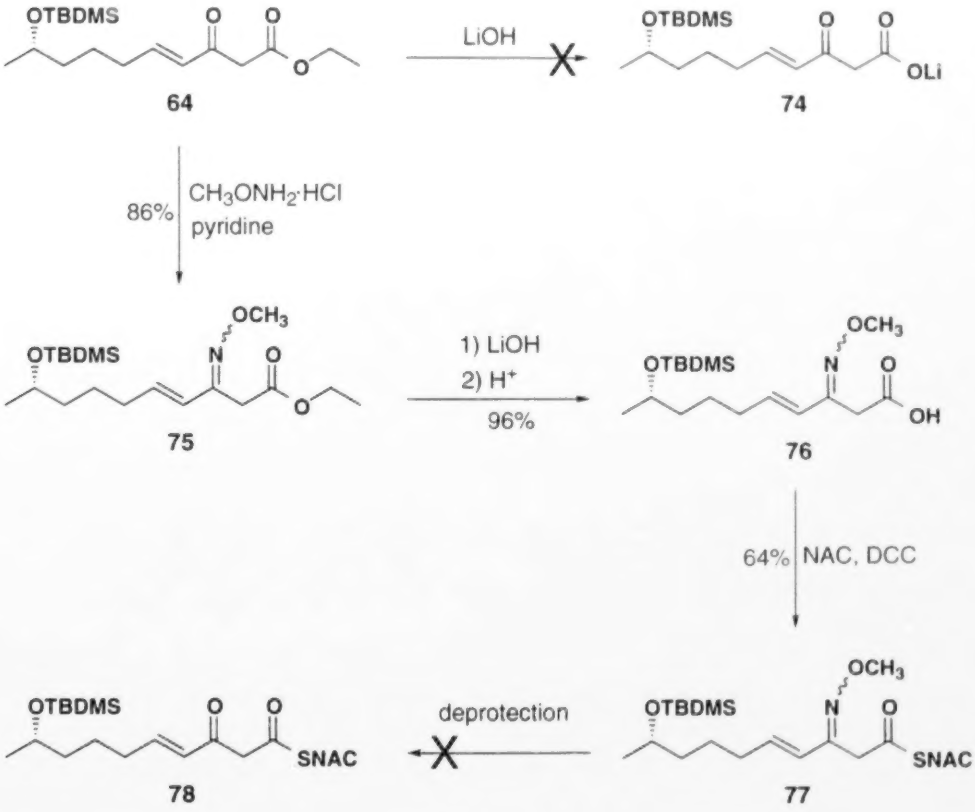


Scheme 20

With the skeleton of the pentaketide **50** completed, the remaining steps are hydrolysis of the ester and condensation of the resulting acid with NAC. Unfortunately the base-catalyzed hydrolysis is problematic, and leads to complex mixtures (Scheme 21). Examination of the process by ^{1}H NMR spectrometry in D_2O/THF-d_8 (v/v 1:2) and D_2O/CD_3OD (v/v 1:2) shows that in both solvent systems the disappearance of the vinyl signals occurs faster than the hydrolysis of the ethyl ester. This suggests that side reactions such as Michael addition, followed by retro-aldol may fragment the molecule.

A plausible solution to this problem is protection of the keto carbonyl before hydrolysis. This also avoids the risk of decarboxylation of the free β-keto acid. Protection as an acetal (1,3-dioxolane) proved difficult, because the silyl group does not survive the acidic conditions. Oxime derivatives appeared as a reasonable alternative because they form readily under basic conditions.[59] Thus, reaction of **64** with *O*-methylhydroxylamine gives *O*-methoxime **75** in good yield, as a mixture of *syn* and *anti* isomers (Scheme 21). Saponification of the ethyl ester proceeds smoothly to afford the acid **76**, which condenses with NAC to generate the corresponding NAC thioester **77**. Unfortunately, the

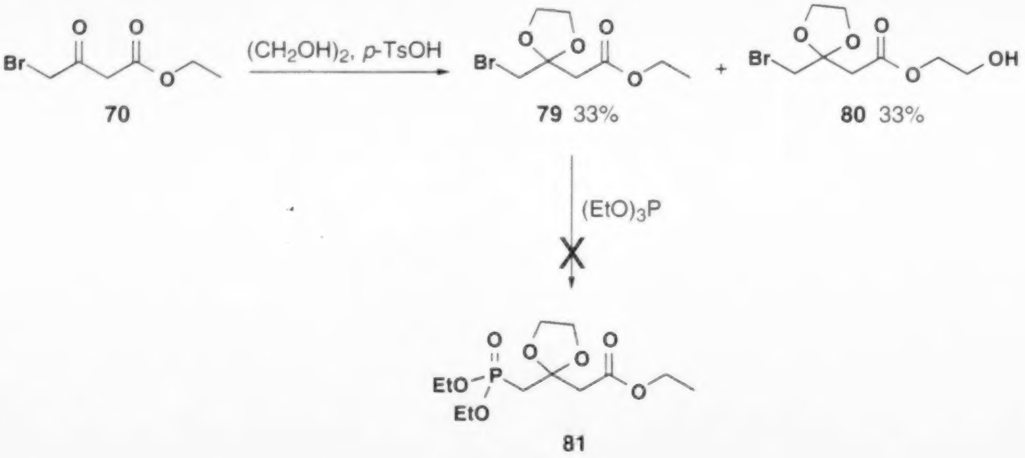
O-methoxime is resistant to deprotection. Treatment with Amberlyst-15 as described by Sakamoto *et al.*[60] results in loss of the silyl group, leaving the oxime untouched. With Corey's titanium reagent made by reduction of $TiCl_3 \cdot 3THF$ with DIBAL in toluene,[59] compound **77** undergoes slow decomposition to form a complex mixture, none of the desired product **78** could be isolated.


OTBDMS
O
O
O
64
LiOH
OTBDMS
O
O
OLi
74
86%
$CH_3ONH_2 \cdot HCl$
pyridine
OCH3
OTBDMS
N
O
O
75
1) LiOH
2) H+
96%
OCH3
OTBDMS
N
O
OH
76
64%
NAC, DCC
OTBDMS
O
O
SNAC
78
deprotection
OCH3
OTBDMS
N
O
SNAC
77

Scheme 21

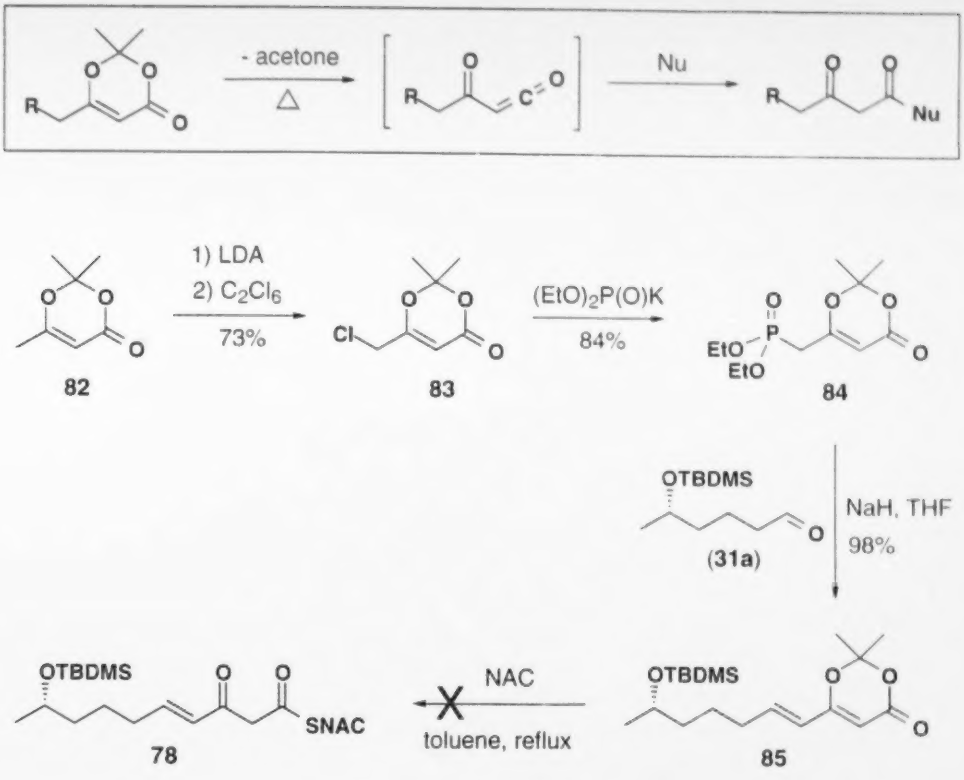
Another option could be to protect the carbonyl at the acetoacetate stage before the introduction of the C-6 unit with the acid-sensitive silyl group. Treatment of bromide **70** with ethylene glycol in the presence of *p*-toluenesulfonic acid (*p*-TsOH) gives acetal **79** with the formation of an equal amount of the transesterification product **80** (Scheme

22). However, acetal **79** is unreactive under the conditions of Arbuzov reaction, presumably due to steric hindrance.



Scheme 22

Diketene-acetone adduct, 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (**82**) has been reported as an effective acetoacetylation reagent.[61] Thermolysis of **82** in the presence of nucleophiles gives β-keto acid derivatives, presumbly *via* a ketene intermediate (Scheme 23). Hence it appeared that the target pentaketide could be made by thermolysis in the presence of NAC to generate the β-keto NAC ester directly. Chlorination of **82** with hexachloroethane gives **83** in good yield, which is then readily converted to phosphonate **84**.[61d] Condensation of aldehyde **31a** with the phosphonate **84** produces the key intermediate **85**. However, the thermolysis of **85** with NAC in refluxing toluene gave complex mixtures and none of the desired target **78** could be isolated.


- acetone
△
Nu
R
Nu
1) LDA
2) C₂Cl₆
73%
(EtO)₂P(O)K
84%
NaH, THF
98%
NAC
toluene, reflux
OTBDMS
SNAC
Cl
EtO
82
83
84
(31a)
78
85

Scheme 23

The attempts to generate **78** clearly demonstrate that the presence of sensitive functional groups and the facile lactonization of intermediates hinder the synthesis of the labeled pentaketide **50**. The need to develop a reliable method for the construction of δ,γ-unsaturated β-ketothioesters, possibly using new protecting groups, still remains.

In summary, the pentaketide ethyl ester **64** can be synthesized by condensation of aldehyde **31a** with a four-carbon phosphonate reagent **73**. However, the NAC ester of the doubly ^{13}C-labeled pentaketide **50** is not easily accessible. Modified NAC derivative **28** has been examined for incorporation by attaching it to the known unsaturated tetraketide precursor, and it is not accepted by the polyketide synthase machinery. Interestingly, studies on the late stage transformations reveal that dehydrocurvularin and

8-hydroxycurvularin are interchangeable in the fermentation culture, with the interconversion being catalyzed by some component(s) from the potato dextrose broth. A number of advanced precursors were synthesized in multiply labeled form. Diketide **7** with keto functionality and saturated tri- and tetraketides **8** and **27** were made bearing two ^{13}C-labels and tested for incorporation into dehydrocurvularin (**1**) by *A. cinerariae*. The results show that the diketide **7** is a precursor in the biosynthetic pathway of dehydrocurvularin, and that the double bond of the tetraketide **5** is essential for incorporation. This strongly supports the hypothesis that the unsaturated tetraketide represents the final oxidation state achieved on the PKS enzyme prior to addition of the next C_2 unit. Since the penta-, hexa-, hepta-, and octaketide PKS intermediates require no reduction (i.e. they remain β-ketothioesters), this suggest that dehydrocurvularin (**1**) is the initial PKS product.

Chapter 2 Modification of the Swern Oxidation

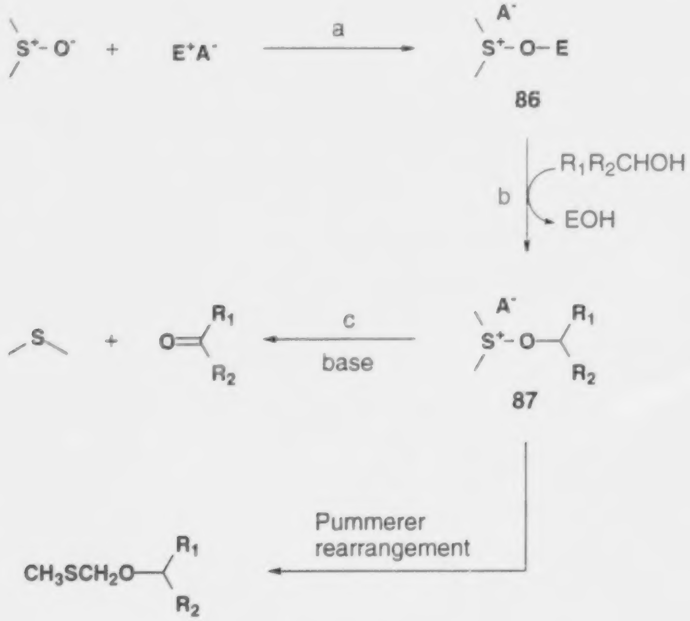
INTRODUCTION

During the biosynthetic studies on dehydrocurvularin (Chapter one) Swern oxidations have been used many times for synthesis of the putative precursors. The work described below aims to improve the reaction and make it more environmentally acceptable.

Oxidations of primary or secondary alcohols to aldehydes or ketones using activated dimethyl sulfoxide (DMSO) are among the most widely used reactions in organic synthesis.[62] A variety of reaction procedures with different activators are commonly employed. They all form carbonyl products in high yields, without major side reactions such as racemization or over-oxidation. The mild reaction conditions mean the reaction is compatible with a wide range of other functional groups.

The activators are normally carbodiimides, acid halides and acid anhydrides. Dicyclohexylcarbodiimide (DCC) is by far the most widely used diimide (Pfitzner-Moffatt procedure[63]); oxalic acid is utilized in the procedure to convert any residual DCC to the highly insoluble urea, which is removed by filtration. The acid halide and anhydride activators most frequently used are oxalyl chloride (Swern procedure[62c]), trifluoroacetic anhydride (TFAA) (Swern procedure[62c]), sulfur trioxide/pyridine complex (Parikh-Doering procedure[64]), and acetic anhydride (Albright-Goldman procedure[65]). Other activation methods include the use of methanesulfonic anhydride, cyanuric chloride, benzoyl chloride, mesyl chloride, tosyl chloride, thionyl chloride, acetyl bromide, acetyl chloride, phosphorus pentoxide and phenyl dichlorophosphate.

All these procedures share a common mechanism proceeding in a sequence of three steps (Scheme 24): a) activation of DMSO by an electrophilic reagent (E^+A^-) to generate **86**; b) attack by an alcohol on the electropositive sulfur atom with the departure of a leaving group to form a salt **87**; c) reaction with a base (typically triethylamine) to produce dimethyl sulfide and the carbonyl product. As a competing reaction the alkoxydimethylsulfonium salt **87** can undergo Pummerer rearrangement to form the by-product methylthiomethyl ether. Fortunately, this side reaction can be minimized by performing the reaction at low temperature.

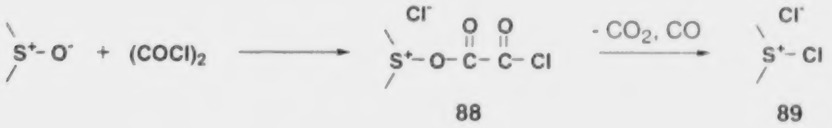


Scheme 24

Activation of DMSO with oxalyl chloride, known as Swern oxidation, is now the most widely used procedure.[66] It often generates high and frequently quantitative yields of the corresponding carbonyl compound; the yields are slightly dependent on the reaction temperature up to -20 °C. To minimize the formation of methylthiomethyl ethers and other by-products, the reaction is normally performed in dichloromethane at -60 °C. Oxalyl chloride also has the advantages of lower cost and toxicity.

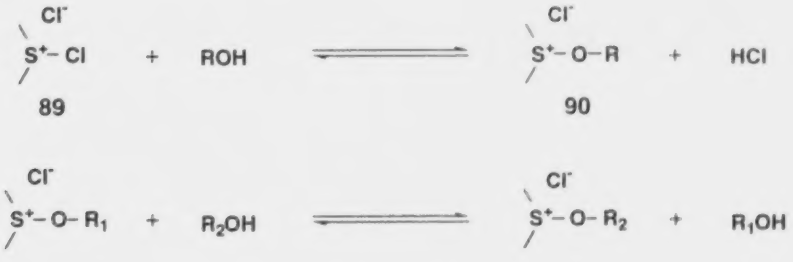
Oxalyl chloride reacts vigorously with DMSO even at -60 °C, resulting in immediate evolution of gas. The intermediate of this activation was carefully studied by

Swern and co-workers.[66a] Low temperature IR (-140 °C) and ^{13}C NMR (-60 °C) spectrometric examinations of the intermediate show no carbonyl group absorptions or carbonyl carbon signals. It was also noticed that only one of the acyl halide functions of oxalyl chloride undergoes displacement by DMSO at -60 °C in dichloromethane. These results clearly imply that the initial adduct **88** collapses rapidly to the chlorodimethylsulfonium species **89** (Scheme 25). The loss of carbon dioxide and carbon monoxide is very favorable thermodynamically, and **88** is not stable even at -140 °C.[66a]



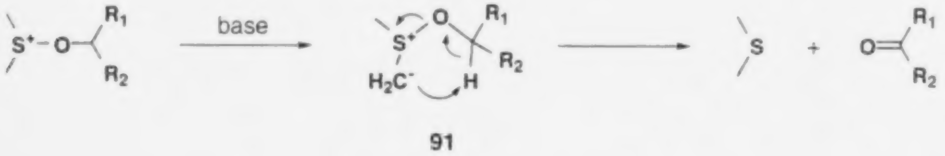
Scheme 25

Based on the results of competitive oxidations, Marx and Tidwell[67] suggested that the reaction of chlorodimethylsulfonium **89** with alcohols is an equilibrium; the resultant alkoxysulfonium salt **90** undergoes exchange with other alcohols (Scheme 26). Oxidation of a mixture of two alcohols added in sequence with less than one equivalent of oxidant shows a preference for oxidation of the first added alcohol, but this effect diminishes with time if the mixture is aged before the addition of triethylamine. These experiments also show preferential oxidation of the less crowded and more electron-rich alcohols.



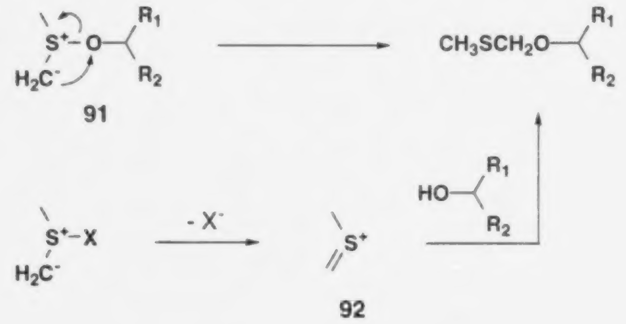
Scheme 26

The chemistry of alkoxysulfonium salts has been studied in some detail. The alkoxysulfonium salts react with base to give the carbonyl product and dimethyl sulfide. Labeling experiments[68] show that the oxidation occurs *via* ylide **91**, which subsequently collapses by an intramolecular cyclic mechanism as shown in Scheme 27. It was also found that alkoxysulfonium salts without hydrogen *alpha* to the positively charged sulfur atom can not undergo the oxidation since the corresponding ylide can not form.[68a]



Scheme 27

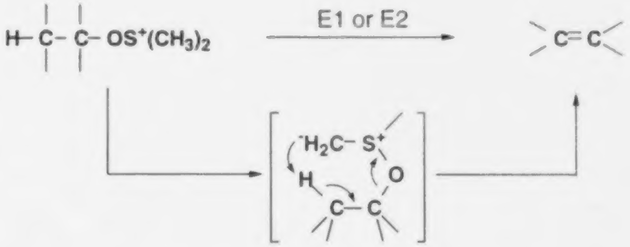
As mentioned earlier the oxidation reactions sometimes compete with the formation of by-product methylthiomethyl ethers *via* Pummerer rearrangement. Two possible mechanisms were proposed:[69] these by-products could arise either *via* an intramolecular rearrangement of the alkoxysulfonium ylide **91**; or by formation of the methylenesulfonium cation **92**, which intermolecularly alkylates the alcohol (Scheme 28). Crossover experiments[69, 70] suggest that intermolecular reactions are involved. If intramolecular rearrangements were the pathway, they would be equally probable in all the activation methods, since **91** is formed in each, but this is not observed.



Scheme 28

Under Swern conditions the oxidation of primary and secondary alcohols to the corresponding carbonyl compounds is general. However, difficulties are encountered with 2-phenylethanol and 2-phenyl-1-propanol[66a] (23% and 38% yield of the aldehydes, respectively) and certain types of alkynyl alcohols.[66b] Side reactions other than the formation of methylthiomethyl ethers may occur in other specific circumstances. These include:

1) *Product isomerization by enolization.* This is a possibility that always exists with the generation of aldehydes and ketones in the presence of bases.
2) *1,2-Elimination to alkenes.* This potential side reaction can occur by the E1 or E2 processes, or *via* alkoxysulfonium ylides, which eliminate DMSO through six-membered transition states as shown in Scheme 29.

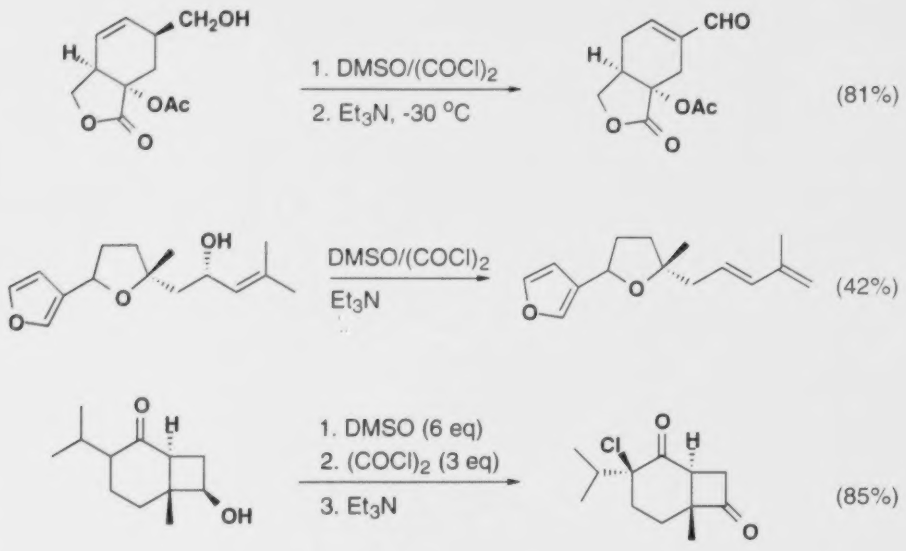


1,2 Elimination of alkoxysulfonium salts

Scheme 29

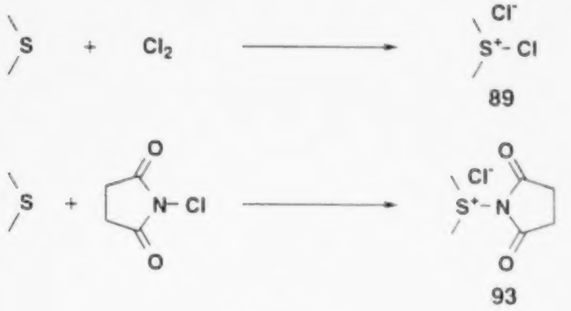
3) *Substitutions.* A potential competition between oxidation and substitution exists for the alkoxysulfonium ions because sulfoxides are good leaving groups. For example, the oxidation of allyl alcohol is usually contaminated by the formation of allyl chloride.
4) *Further reactions of the carbonyl products.* The carbonyl products may undergo cyclization and dehydration, or may react with excess DMSO/oxalyl chloride to give α-chloro ketones.

Representative examples[71] of these side reactions are given in Scheme 30.



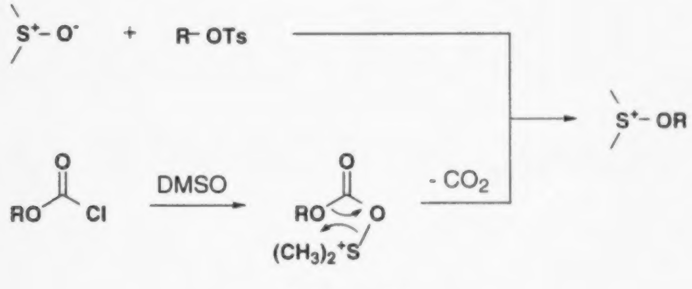
Scheme 30

Reaction of dimethyl sulfide with halogens and halosuccinimides forms similar *S*-halosulfonium species which can induce oxidation reactions.[72] Electrochemical oxidation[73] of sulfides ($RSCH_3$, R = CH_3, n-C_8H_{17}, C_6H_5) has been reported as the activating step for the conversion of secondary alcohols to ketones. Corey and Kim found that dimethyl sulfide reacts with chlorine to produce the same intermediate as in the Swern oxidation, but the procedure is not as convenient as the latter (Scheme 31). For laboratory oxidations, they reported the reaction of dimethyl sulfide with *N*-chlorosuccinimide (NCS) to give imidodimethylsulfonium complex **93**, which conveniently converts alcohols to the corresponding aldehydes or ketones (Scheme 31). A polymer-supported sulfide reagent[74] has also been used with chlorine, but the yield of the carbonyl product using this reagent is not remarkable, e.g. 67% for the conversion of benzyl alcohol to benzaldehyde.



Scheme 31

Another related process is reaction of DMSO with primary alkyl tosylates[75] or alkyl chloroformates[76] which are obtained by treatment of alcohols with phosgene. The reactions involve a nucleophilic displacement of the tosylate or chloroformate function by DMSO to form the intermediate alkoxysulfonium salt, which produces a carbonyl compound and dimethyl sulfide upon treatment with base. Activation of DMSO by chloroformate esters is proposed to occur as shown in Scheme 32. The mechanism implies that the oxygen of the alcohol is retained in the product. These reactions demonstrated the nucleophilicity of the oxygen atom of DMSO, which was estimated by Smith and Winstein[77] to exceed that of ethanol towards alkanesulfonate esters.



Scheme 32

RESULTS AND DISCUSSION

1. A Liquid Version Modification

As discussed in the introduction, the Swern oxidation employing DMSO and oxalyl chloride is the most effective of all the activation procedures, but the process typically uses excess reagent and produces the unpleasant-smelling volatile (bp 37 °C) by-product, dimethyl sulfide. It is desirable to make a modification which eliminates the smell of the by-product and makes the reagent recyclable. The initial idea was to design a molecule with a structure as shown in Figure 15, which has a functionality attached to the sulfoxide group through a spacer. This functionality serves as a "handle" to allow easy separation of the reagent after the reaction, but remains inert throughout the Swern oxidation. A carboxylic acid possesses all the features of such a group. It is stable to the oxidation conditions, and its polar properties and solubility in basic aqueous solution offer feasible separation from normal organic compounds.

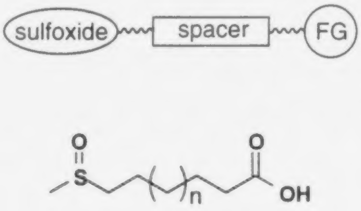
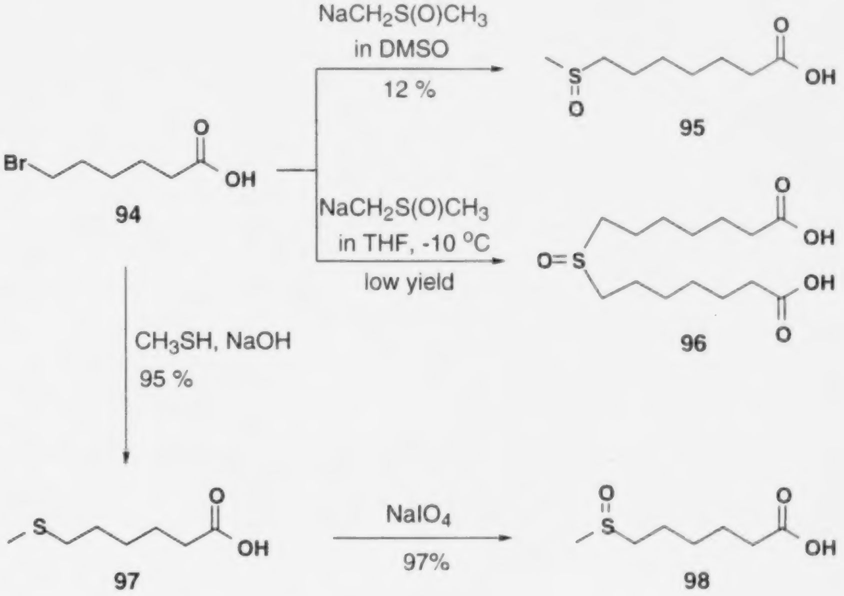


Figure 15

Synthetically this type of compound can be easily constructed by nucleophilic substitution of ω-halocarboxylic acid with dimsyl sodium (sodium methylsulfinyl carbanion) or methanethiol. Consideration of the stench of methanethiol initially gives preference to the route using dimsyl sodium. Dimsyl sodium solution[78] is generated by warming a suspension of sodium hydride in DMSO. Treatment of commercially available 6-bromohexanoic acid (**94**) with 1.5 equiv of such a solution at room temperature gives the expected product **95** in only 12% yield (Scheme 33). Reaction with excess (3 equiv) of dimsyl sodium in THF at lower reaction temperature results in incomplete reaction, and forms the dialkylated product **96** in low (ca. 15%) yield. Alternatively, reaction of **94** with methanethiol[79] in methanol in the presence of sodium hydroxide affords the sulfide **97** in 95% yield. Subsequent oxidation to the sulfoxide **98** with sodium metaperiodate[80] proceeds almost quantitatively without any significant over-oxidation to the corresponding sulfone.



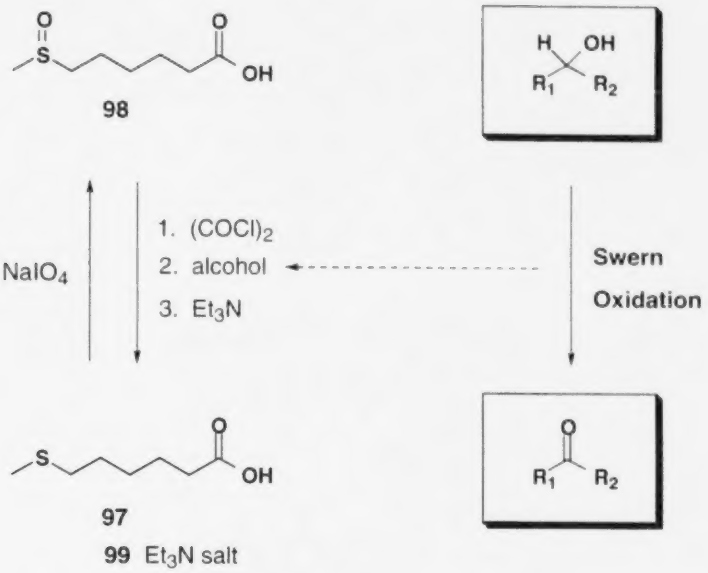
Scheme 33

In order to test the efficacy of **98** as a substitute for DMSO in the Swern oxidation reaction, a number of alcohols were oxidized by this reagent and oxalyl chloride under standard Swern conditions. In a typical small scale procedure, a dichloromethane solution of **98** in slight excess (1.2 equiv) is treated at -50 to -60 °C with oxalyl chloride (1.1 equiv) followed by a solution of the alcohol (1.0 equiv), and finally, excess triethylamine (5 equiv). The reaction mixture is allowed to stir for 5 min at -60 °C and is then warmed to room temperature. After the silica gel filtration, the product was analyzed by ^{1}H NMR spectrometry using a long relaxation delay (12 s). The alcohol/carbonyl ratio was determined by normalizing the integrations of well-resolved signals. The results are summarized in Table 1. This new procedure often leaves a small amount of unreacted alcohol (7-9%).

Table 1 Oxidation of alcohols with standard Swern conditions using **98**

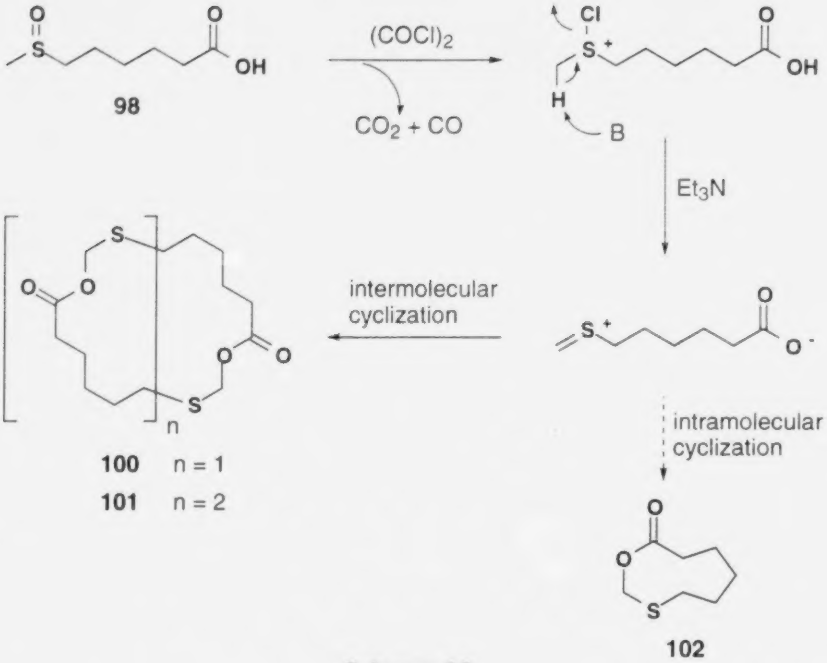
Alcohol	Recovered Yield (%)	Product Composition (%)	
		R_1R_2CO	R_1R_2CHOH
endo-Borneol	93	91	9
3,5-Dimethoxybenzyl alcohol	97	93	7
n-Dodecanol	95	92	8
Cinnamyl alcohol	95	92	8

Interestingly the carboxyl group does not interfere in the oxidation reaction, despite the potential to form acid chloride or react with the activated sulfonium intermediate (see below). After the reaction the sulfide by-product is present as the triethylammonium salt **99**, which is easily separable from the reaction mixture by a simple filtration through a column of silica gel (Scheme 34). The sulfide **97** is then recovered in >95% yield by acidification of the washed column, and can be readily recycled by aqueous periodate oxidation. Alternatively, for larger scale reactions, the carboxylic acid functionality in the reagent allows a convenient separation of products from **97** by acid-base extraction. Although methanethiol is toxic and has an even more obnoxious stench than dimethyl sulfide, preparation of one large batch of **97**, which is odorless, can suffice for many subsequent Swern procedures.



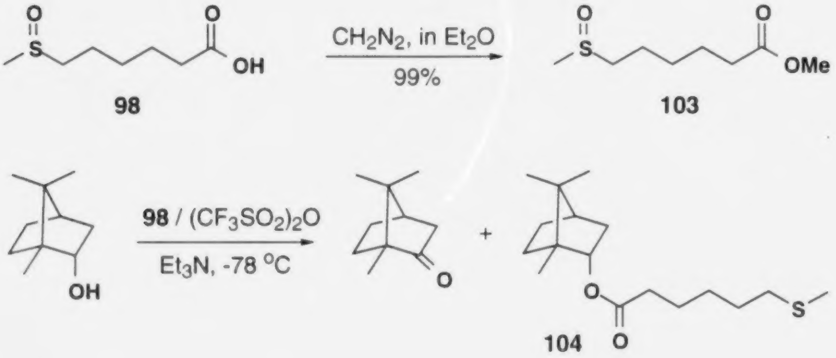
Scheme 34

Use of a two fold excess of oxidizing reagents gives no improvement, and results in contamination by other products. In order to identify the by-products caused by using an excess of reagents, a control experiment (same procedure as above, but without alcohol) was performed. Two products were isolated in yields of 13% and 7% respectively, their structures were elucidated by spectroscopic methods as **100** and **101** (Scheme 35). Presumably they form by intermolecular Pummerer rearrangement. It is interesting to note that only polymeric Pummerer adducts (e.g. **100** and **101**) are isolated and direct intramolecular cyclization to generate a monomer **102** is not detected.


98
(COCl)$_2$
CO$_2$ + CO
Cl
S$^+$
OH
H
B
Et$_3$N
intermolecular cyclization
S$^+$
O$^-$
intramolecular cyclization
n
100 n = 1
101 n = 2
102

Scheme 35

To improve the efficacy of this modified process, various conditions were examined with *endo*-borneol as substrate, and the results are summarized in Table 2. To elucidate the influence of the carboxyl group on the oxidation, the methyl ester **103** was prepared by treating **98** with diazomethane (Scheme 36). Essentially identical results are obtained when **103** is used in place of **98** in the Swern oxidation (entry 2).


98
CH$_2$N$_2$, in Et$_2$O
99%
OMe
103
98 / (CF$_3$SO$_2$)$_2$O
Et$_3$N, -78 °C
OH
+
104

Scheme 36

Different activators were then tested (entries 3-5) under standard conditions. Trifluoroacetic anhydride, an alternative activator developed by Swern *et al.*,[81] gives a similar outcome. Oxalyl bromide leaves more unreacted alcohol. A much stronger activator, triflic anhydride,[82] generates a dark reaction mixture, even at -78 °C, from which 45% carbonyl product, along with **104** could be isolated (Scheme 36). Change of base (entries 6, 7) does not show any improvement. Finally, it was clear that control of temperature and exposure time to triethylamine is important for oxidation efficiency (entries 8-10). It is necessary to maintain stirring at -40 °C for 2 h (or at -60 °C for 4 h) after triethylamine addition to obtain optimum results.

Table 2 Oxidation results under different conditions

Entry	Activator	Base	Temperature[a] (time)	Product Composition (%)	
				Camphor	*endo*-Borneol
1	$(COCl)_2$	Et_3N	-60 °C (5 min)	91	9
2[b]	$(COCl)_2$	Et_3N	-60 °C (5 min)	91	9
3	$(CF_3CO)_2O$	Et_3N	-60 °C (5 min)	87	13
4	$(CF_3SO_2)_2O$	Et_3N	-78 °C (5 min)	45[c]	--
5	$(COBr)_2$	Et_3N	-60 °C (5 min)	80	20
6	$(COCl)_2$	$Et(i\text{-}Pr)_2N$	-60 °C (5 min)	81	19
7	$(COCl)_2$	Me_3N	-60 °C (5 min)	73	27
8	$(COCl)_2$	Et_3N	-20 °C (3 h)	92	8
9	$(COCl)_2$	Et_3N	-40 °C (2 h)	96	4
10	$(COCl)_2$	Et_3N	-60 °C (4 h)	96	4

[a] After the addition of base the mixture was kept at this temperature for a certain time period before warming up to room temperature. [b] The methyl ester **103** was used instead of **98**. [c] Isolated yield.

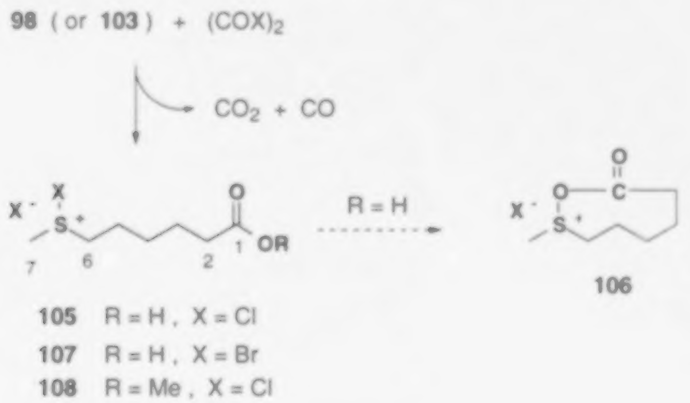
With this improvement a number of alcohols were re-examined with the modified procedure. As shown in Table 3, the amount of unreacted alcohol can be minimized to 1-4% (determined by ^{1}H NMR spectrometry as described above). A portion of the product was further purified by flash chromatography to determine the isolated yields, which are comparable with those of the original Swern reaction reported in the literature. For phenethyl alcohol the isolated yield is higher than that previously described.

Table 3 Oxidation of alcohols with modified Swern procedure using **98**.

Alcohol	Product Composition (%)		Isolated R_1R_2CO Yield (%)	Literature Yield (%)[a]
	R_1R_2CO	R_1R_2CHOH		
endo-Borneol	95	5	90	98
3,5-Dimethoxybenzyl alcohol	96	1	94	--
n-Dodecanol	96	4	92	99
Cinnamyl alcohol	97	3	93	98
Benzoin	97	3	94	95
Phenethyl alcohol	44	33	31[b]	23

[a] See ref 62c. [b] Recovered alcohol 28%.

Low temperature (-60 °C) ^{13}C NMR spectrometry provides information about the intermediates of this modified Swern oxidation (Scheme 37, Table 4). Reaction of **98** with oxalyl chloride at -60 °C rapidly generates the chlorosulfonium salt **105**, whose spectrum exhibits an expected downfield shift for the carbons adjacent to sulfur: $\Delta\delta$ 10.72 ppm for C-7 and 13.44 ppm for C-6. The corresponding difference for the dimethyl chlorosulfonium intermediate derived from DMSO is $\Delta\delta$ 8.5 ppm.[66a] Although the carboxyl carbon also displays a considerable downfield displacement ($\Delta\delta$ 4.49 ppm), the cyclized form **106** can be excluded as a principal component, because oxalyl bromide gives a different intermediate (i.e. bromo analogue **107**) based on the chemical shifts for carbons adjacent to sulfur (upfield shifts were observed due to the lower electronegativity of Br). Moreover, the carboxyl carbon resonance of **107** is shifted similarly to that in **105**. The change in carboxyl shift can be tentatively attributed to disruption of a possible hydrogen bond between the sulfoxide and the acid moiety, based on its absence during conversion of the corresponding methyl ester **103** to its chlorosulfonium analogue **108** with oxalyl chloride.



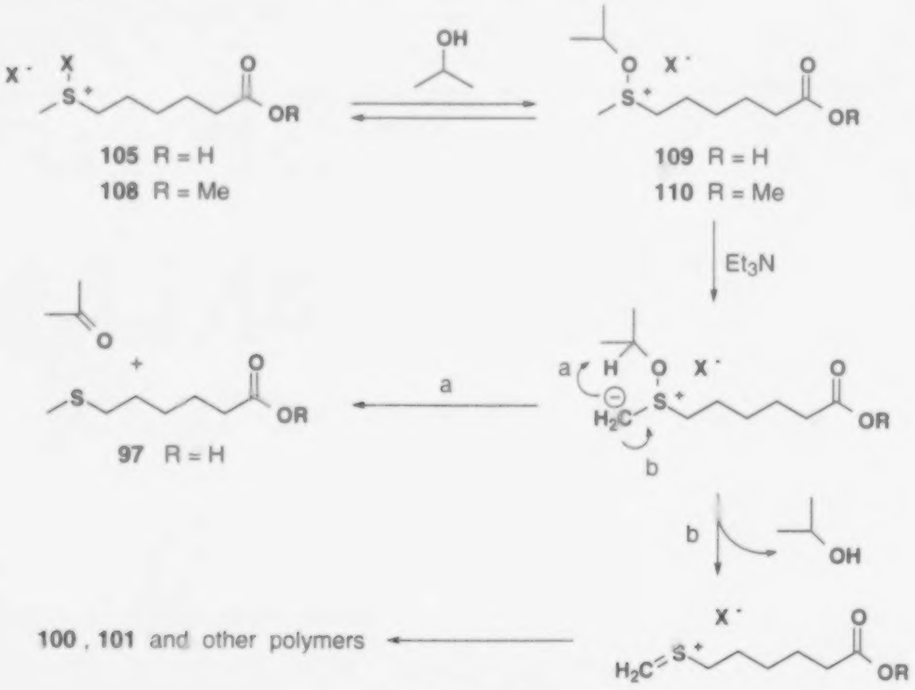
Scheme 37

Table 4 ^{13}C NMR data at -60 °C (50 MHz, CD_2Cl_2)

Compound[a]			Chemical Shift			
			C-7	C-6	C-2	C-1
98	R = H		36.92	52.32	33.22	176.31
105	R = H,	X = Cl	47.64	65.76	33.25	180.80
107	R = H,	X = Br	25.93	43.78	33.22	180.77
103	R = Me		38.05	51.54	33.11	173.81
108	R = Me,	X = Cl	47.63	65.78	32.99	173.95

[a] For structures see Scheme 37. Compound **106** was not detected in any experiment.

Elegant studies by Tidwell and coworkers[67] using competitive oxidation experiments indicate than an equilibrium exists between the alkoxydimethylsulfonium intermediates and the chlorodimethylsulfonium species in the usual Swern oxidation (i.e. DMSO). An analogous mechanism could be verified for the reaction of **98** by addition of varying amounts of 2-propanol (0.5 to 2 equiv) to the chlorosulfonium intermediate **105** at -60 °C, with ^{13}C NMR observation of the resulting mixtures of unreacted **105** as well as alkoxysulfonium product **109** (Figure 16). Low temperature addition of a solution of a different chlorosulfonium species **108** consumes the free alcohol and regenerates **105** to unambiguously confirm the existence of the equilibrium (Scheme 38). Since excess oxidizing reagent does not improve the efficiency, the observed dependence of yields on reaction temperature suggests two competing pathways after the addition of base, namely proton transfer from the alkoxy moiety with elimination to give the desired carbonyl compound (route a) and α,β-elimination to a methylenesulfonium cation enroute to Pummerer products.



Scheme 38

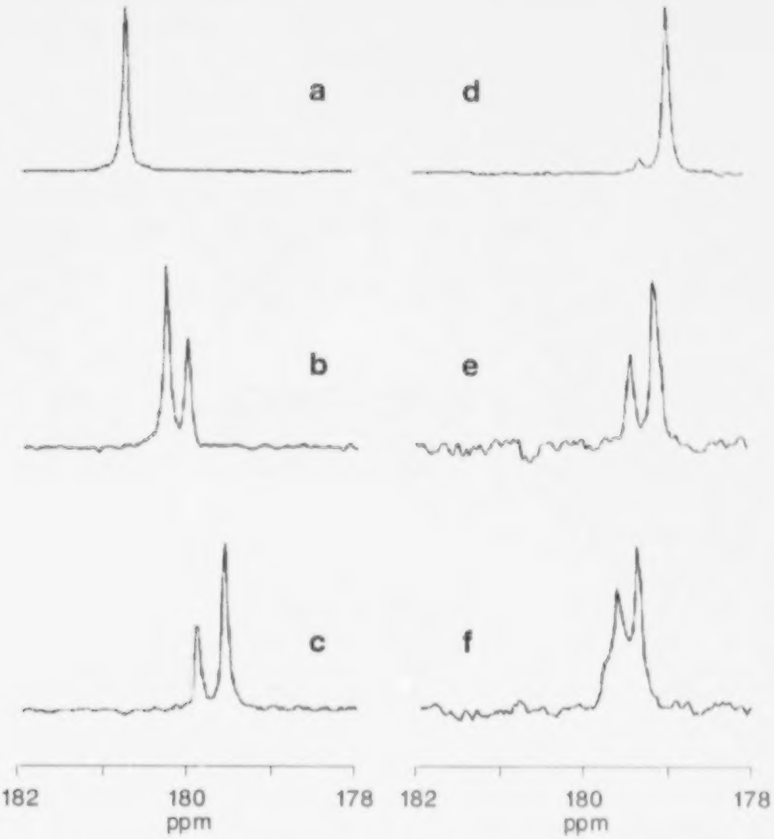


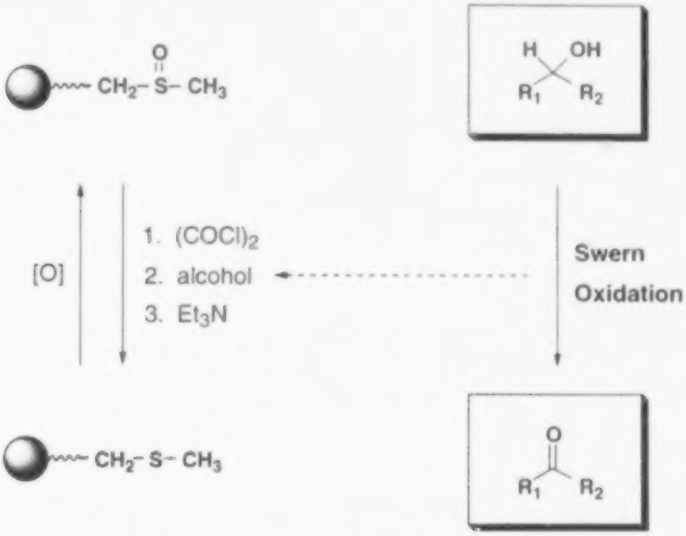
Figure 16. ^{13}C NMR (50 MHz, CD_2Cl_2, -60 °C) spectra of the carboxyl region for the reaction of **105** with 2-propanol. The upfield signal is for product **109**; the carboxyl carbon of **108** (δ 174 ppm) is not shown: a) **105** only; b) **105** + 0.5 eq. 2-propanol; c) **105** + 1.0 eq. 2-propanol; d) **105** + 2.0 eq. 2-propanol; e) **105** + 2.0 eq. 2-propanol + 0.75 eq. **108**; f) **105** + 2.0 eq. 2-propanol + 1.0 eq. **108**.

In summary, readily available 6-(methylsulfinyl)hexanoic acid (**98**) can be employed as a substitute for DMSO in the Swern oxidation to smoothly convert primary or secondary alcohols to corresponding aldehydes or ketones in high yield. The resulting 6-(methylthio)hexanoic acid (**97**) is easily separable by aqueous extraction or by filtration through silica gel, and can be reoxidized to **98** with sodium metaperiodate in 97% yield.

Low temperature (-60 °C) ^{13}C NMR spectrometry has been used to examine the intermediates of this Swern process. The results indicate that any residual unoxidized alcohol is generated during Pummerer elimination of the alkoxysulfonium intermediate and can be minimized by extended exposure to triethylamine at -40 °C.

2. Polymer-Supported Swern Oxidation

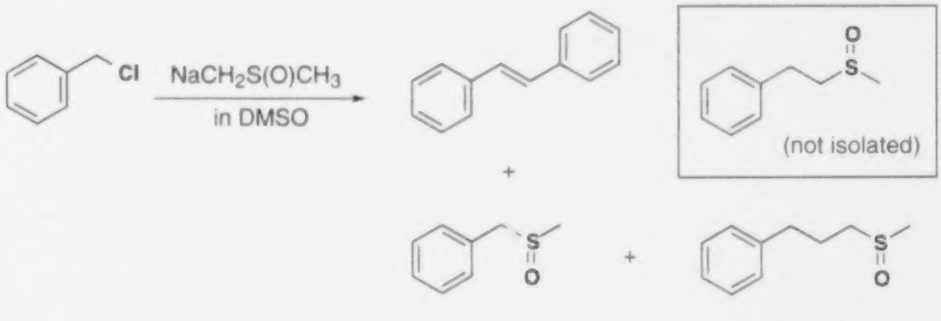
An alternative approach to the modification of the Swern oxidation is to immobilize the sulfoxide functionality on a polymer matrix. After the reaction, the polymer-supported reagents can be easily separated from the low molecular weight compounds by filtration.[83] Separation is easiest if the polymer is cross-linked and in the form of beads at least 50 μm in diameter. Since the reagents are polymer-bound and non-volatile, they are odorless and non-toxic, which makes the reaction more environmentally acceptable. The recovered reagents can possibly be regenerated by oxidation and reused (Scheme 39).



Scheme 39 Polymer-supported Swern oxidation

Commercially available chloromethyl polystyrene resin (1% crosslinked), also known as Merrifield resin, has been chosen as the supporting polymer, because of its mechanical stability and swelling properties in organic solvents. This choice also enables us to take advantage of the well-established chemistry developed for solid state peptide synthesis.[84] The pendant chloromethyl group allows facile nucleophilic attack of sulfur-containing reagents to introduce the required sulfoxide functionality. Because of their simplicity, dimsyl sodium and methanethiol were the first reagents we examined for loading onto the polymer matrix.

Initially benzyl chloride was used as a model to mimic the chloromethyl polystyrene resin, since low molecular weight compounds are easy purify and to characterize using standard techniques. The observations made can then be used to assist analysis of the chemical reactions that occur with the polymer, although the chemical modification of a polymer can not be assumed to exactly mimic the reaction of small molecules. The condensation of benzyl chloride with dimsyl sodium has previously been investigated by Entwistle *et al.*,[85] and was found to be unexpectedly complex. Instead of the expected phenethyl methyl sulfoxide, the reaction products were *trans*-stilbene and a mixture of several other sulfoxides, including benzyl methyl sulfoxide (**111**) and 3-phenylpropyl methyl sulfoxide (**112**) (Scheme 40). We re-examined this reaction. When a solution of benzyl chloride in DMSO is treated with dimsyl sodium at room temperature, a dark purple solution results. Separation of the products gives two fractions, *trans*-stilbene (24% yield) and a mixture of sulfoxides (37% yield, assuming the average molecular weight as that of phenethyl methyl sulfoxide). The sulfoxide fraction shows several spots on tlc with similar Rf's, the two major components were identified as **111** and **112**. As has been noted by other workers,[85, 86] phenethyl methyl sulfoxide could not be isolated.

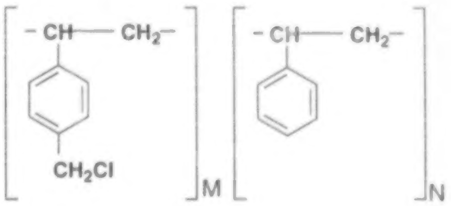


111

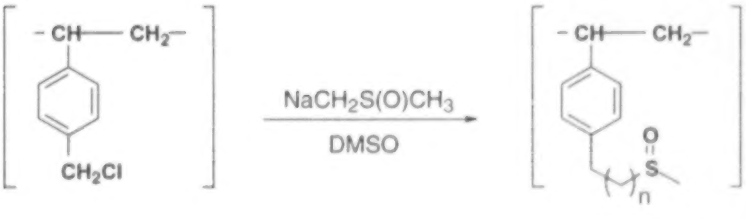
112

Scheme 40

Despite the complexity of this reaction, it appeared that polymer-bound analogues of sulfoxides **111** and **112** could be useful reagents for the Swern process. Hence, commercial chloromethyl polystyrene resin **113** (4.15 mequiv/g, Bio-beads S-X-1, from Bio-Rad) was washed with dichloromethane and dried under high vacuum at 60 °C. Elemental analysis confirmed the chlorine content and indicated that the structure of **113** can be represented as a random repetition of two monomer units, *p*-chloromethylstyrene and styrene, with M/N = 0.85 (Scheme 41). For convenience, the styrene unit will be ignored in all the diagrams of the polymer structure.



Chloromethyl Polystyrene Resin (**113**)



113

114

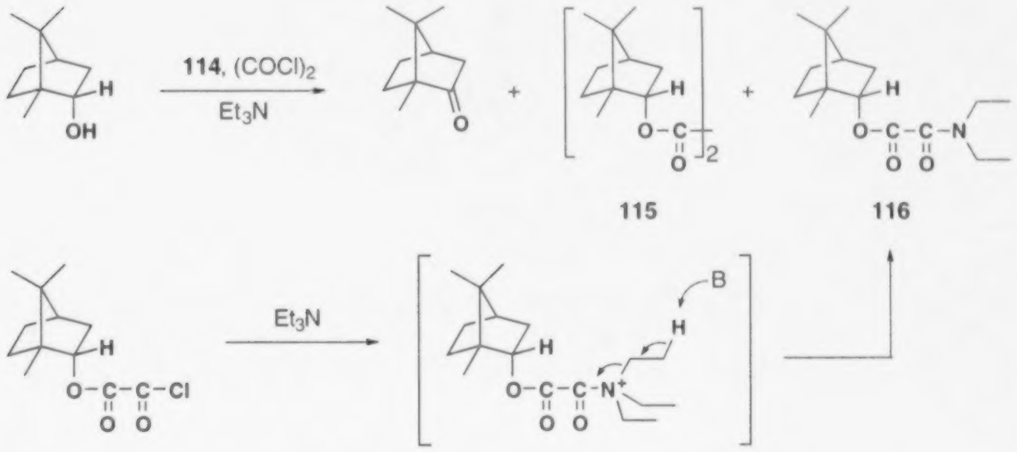
Scheme 41

Reaction of **113** with dimsyl sodium under similar conditions to those employed with benzyl chloride gives the same color change. After aqueous workup, the resin **114** shows a light yellow color, its IR spectra displays a strong band for sulfoxide stretching at 1056 cm^{-1}, and the absorption at 1266 cm^{-1} corresponding to the CH_2Cl group in **113** disappears. The content of sulfur is 5.7 ± 0.3 % by elemental analysis. Comparison to the calculated value based on complete conversion shows that there is approximately 50% sulfur substitution. By analogy to the reaction of benzyl chloride, resin **114** can be assumed to be a mixture of sulfoxides with different chain lengths. Cross-linking between benzyl groups may also occur.

Quantitatively determining the exact state of functionalization of the polymer support is often difficult due to the shortcomings of the analytical methods used. Mass recovery of resin is only at best a rough estimate of the extent of reaction, since the resin has a tendency to stick to untreated glassware. Elemental analyses of H, N, S and X are usually consistent with other estimates, but the determination of carbon is frequently variable. The content of conversion involving polymers could be estimated reasonably from elemental analysis results for sulfur.

Low crosslinked (1-2%) polystyrenes have no permanent pores and swell extensively in certain solvents. The functional groups attached to these polymers have high reactivities and excellent mobilities in the swollen state, and their ^{13}C NMR spectra can be measured easily, albeit with some line broadening, under conventional conditions,[87] although their ^{1}H NMR spectra usually lack fine structure and are often useless for analytical purposes. Resin **113** in CD_2Cl_2 exhibits two sharp peaks at 46.88 and 40.88 ppm in addition to the broad aromatic signals. The peak at 46.88 ppm is assigned to the chloromethyl group, and that at 40.88 ppm is probably due to the methylene moiety on the polystyrene backbone. However, the product resin **114** bearing sulfoxide groups does not show any useful signals in its ^{13}C NMR spectrum even after a large number of scans. This can probably be explained by the side reactions such as *trans*-stilbene formation observed in the reaction of benzyl chloride, which extensively cross-link the polymer and make the structure rigid.

With the polymer reagent **114** available, its oxidation capacity could be studied. Oxidation capacity is the percentage of the total amount of sulfur which can be accessed for Swern oxidation, or alternatively, the numbers of millimoles of alcohol each gram of polymer reagent can oxidize. Oxidation of *endo*-borneol with **114** (1 equiv, based on total sulfur content), oxalyl chloride (1.2 equiv) and excess triethylamine gives a mixture of camphor, unreacted *endo*-borneol, its oxalate **115** and compound **116** (Scheme 42). The percentage of camphor is 50% by ^{1}H NMR integration, which suggests that 50% of the total sulfur is active for the oxidation of *endo*-borneol under Swern conditions. The formation of **116** probably arises from an elimination reaction of the adduct of triethylamine and oxalic monoester (Scheme 42).



Scheme 42

A number of alcohols were examined for Swern reaction using this reagent at 75% of its oxidation capacity (Table 5). The oxidation employed the standard Swern procedure except that the polymer was swollen for 15 min before the addition of oxalyl chloride, and the solution was stirred longer after each of the reagents was added to ensure the accessibility of reagents to the reaction sites. The crude product was analyzed by ^{1}H NMR spectrometry (RD = 12 s) to determine the ratio of the carbonyl compounds to unreacted alcohols. For small alcohols, such as *n*-octanol and benzyl alcohol, the oxidation is complete without any detectable unreacted alcohols. Increasing the size of the alcohols results in incomplete reaction; 6% and 8% of alcohol is left unreacted for *endo*-borneol and *n*-dodecanol, respectively. Interestingly, in the case of 3,5-dimethoxybenzyl alcohol and cholesterol, no reaction is observed. Presumably these two compounds are too large to enter the pores and reach the reactive sites of the polymer reagent under these reaction conditions.

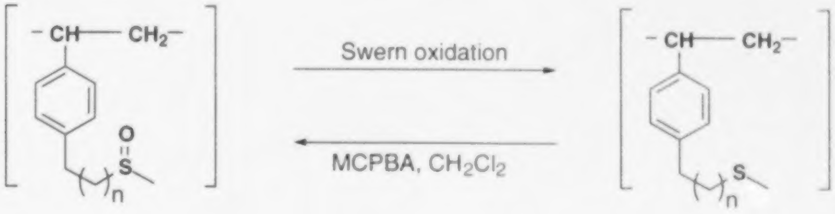
Table 5 Oxidation of alcohols using **114** at 75% of its capacity

Alcohol	Product Composition (%)	
	R_1R_2CO	R_1R_2CHOH
n-Octanol	100	0
n-Dodecanol	92	8
Benzyl alcohol	100	0
endo-Borneol	94	6
Cinnamyl alcohol	82*	0
3,5-Dimethoxybenzyl alcohol	0	100
Cholesterol	0	100

* Cinnamyl chloride is presumably the by-product.

Reagent **114** was tested for regeneration and the possibility of reuse (Scheme 43). After the first cycle of Swern reaction using the procedure as described for the determination of oxidation capacity, the fully reduced polymer reagent **117** was washed with dichloromethane and dried. IR spectroscopy of the recovered polymer did not show any sulfoxide absorption around 1050 cm^{-1}. Treatment of the recovered resin with one equivalent (estimated by the amount of alcohol oxidized in the first cycle) of *m*-chloroperbenzoic acid (MCPBA) in dichloromethane regenerates the reagent, which shows a moderate absorption band at 1054 cm^{-1}. The regenerated polymer is capable of

Swern oxidation, however its oxidation capacity on *endo*-borneol decreases from the initial 50%, based on sulfur content (S), to 36% of S, whereas the total amount of sulfur determined by elemental analysis does not change significantly (Table 6).



114 **117**

Scheme 43

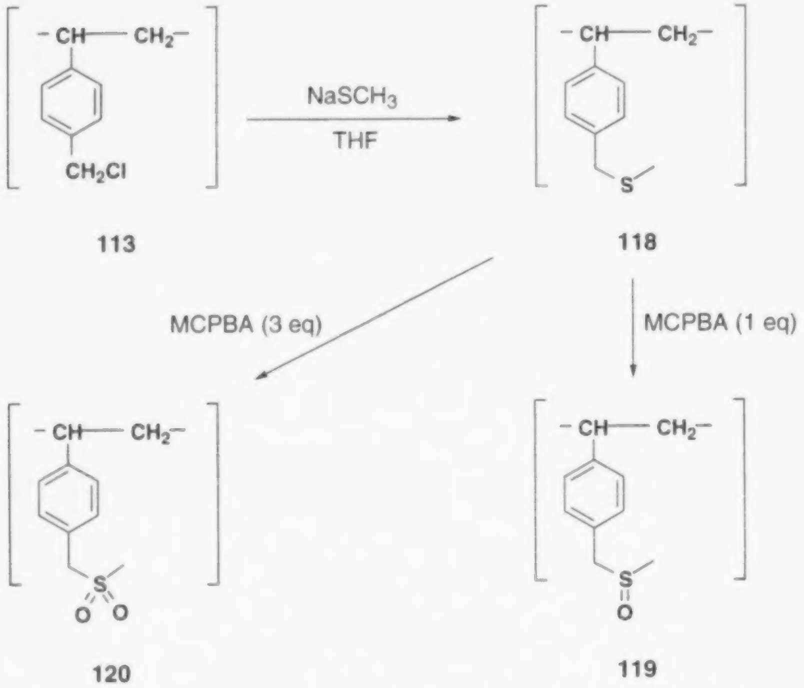
Table 6 Regeneration of polymer reagent **114**

Cycle #	Polymer	Elemental Analysis			Oxidation Capacity
		C%	H%	S%	
1	Sulfoxide	82.47	7.43	5.68	50% of S (0.89 mmol/g)
	Sulfide	84.01	7.52	5.61	
2	Sulfoxide	82.13	7.15	5.45	36% of S (0.61 mmol/g)
	Sulfide	83.63	7.65	5.19	

If the recovered polymer from the second oxidation is regenerated for another (the third) cycle, its sulfoxide IR absorption is much less intense, and therefore no further test was performed. This loss of activity may be attributed to the side reactions in the Swern oxidation or the inefficient conversion of sulfide to sulfoxide during the regeneration. Any side reactions which occur on the polymer reagent may result in the permanent attachment of functionalities to the polymer backbone which block the desired reactions.

The inherent complexity of the dimsyl sodium reaction with benzylic halides and the problems encountered with the corresponding functionalized polystyrene led us to investigate alternative polymers.

Treatment of chloromethyl polystyrene resin **113** in THF with excess sodium thiomethoxide,[85] prepared from methanethiol gas and sodium hydride, provides polymer **118** (Scheme 44). The sulfide **118** shows a clear ^{13}C NMR spectrum, with two sharp peaks at 38.76 and 15.07 ppm for the pendant methylene and methyl groups, in addition to the signals for the polystyrene backbone. The substitution is almost complete since the resonance of the residual CH_2Cl is insignificant, and the sulfur content determined by elemental analysis matches the calculated value.



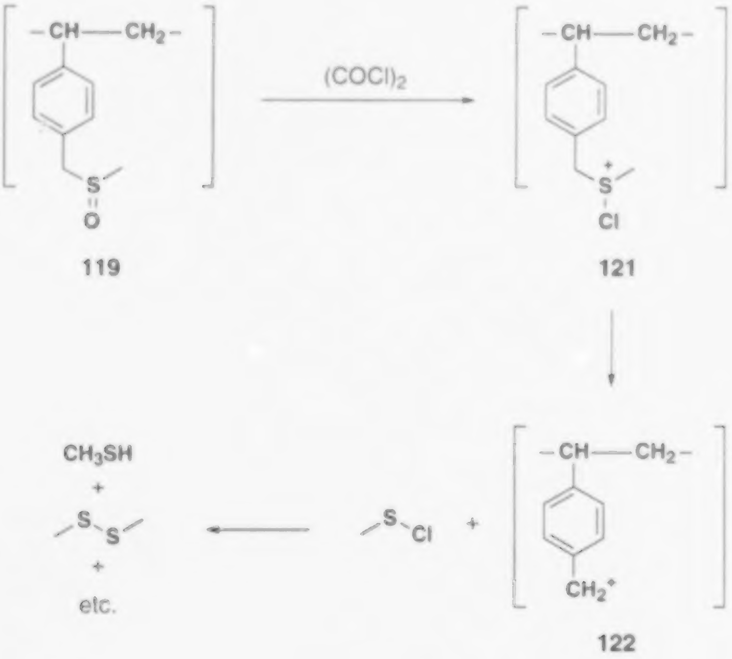
Scheme 44

Oxidation of **118** with one equivalent of MCPBA[89] affords the sulfoxide **119**. Interestingly, its IR shows a broad band with moderate intensity at 3439 cm^{-1} in addition to sulfoxide absorption at 1054 cm^{-1}, probably due to water bound by the sulfoxide functionality. The resultant polymer shows a very weak ^{13}C NMR spectrum. The signal to noise ratio is greatly improved by azeotropic water removal from the polymer with refluxing benzene (in a Soxhlet extractor with CaH_2 trap). The resonances of the methylene and methyl appear at 60.82 and 37.92 ppm, respectively. The presence of an extra peak at 39.56 ppm, though small, suggests the possibility of some over-oxidation to sulfone **120**.

It is known that MCPBA can oxidize sulfide to sulfoxide or sulfone depending on the amount used.[90] It is likely that the polymer sulfoxide is contaminated with sulfone even though one equivalent of oxidant is used, especially since the reaction is not homogeneous. As a reference, polymeric sulfone **120** was prepared by treating **118** with excess MCPBA. Azeotropically dried **120** shows signals at 61.24 and 39.62 ppm, which confirms the sulfone formation side reaction in the preparation of **119**. Benzyl methyl sulfone was tested for Swern oxidation to replace DMSO, but was found to be inert. Although the sulfone does not interfere with the Swern oxidation, it does decrease the oxidation efficiency, as observed in the regeneration of polymer **114**.

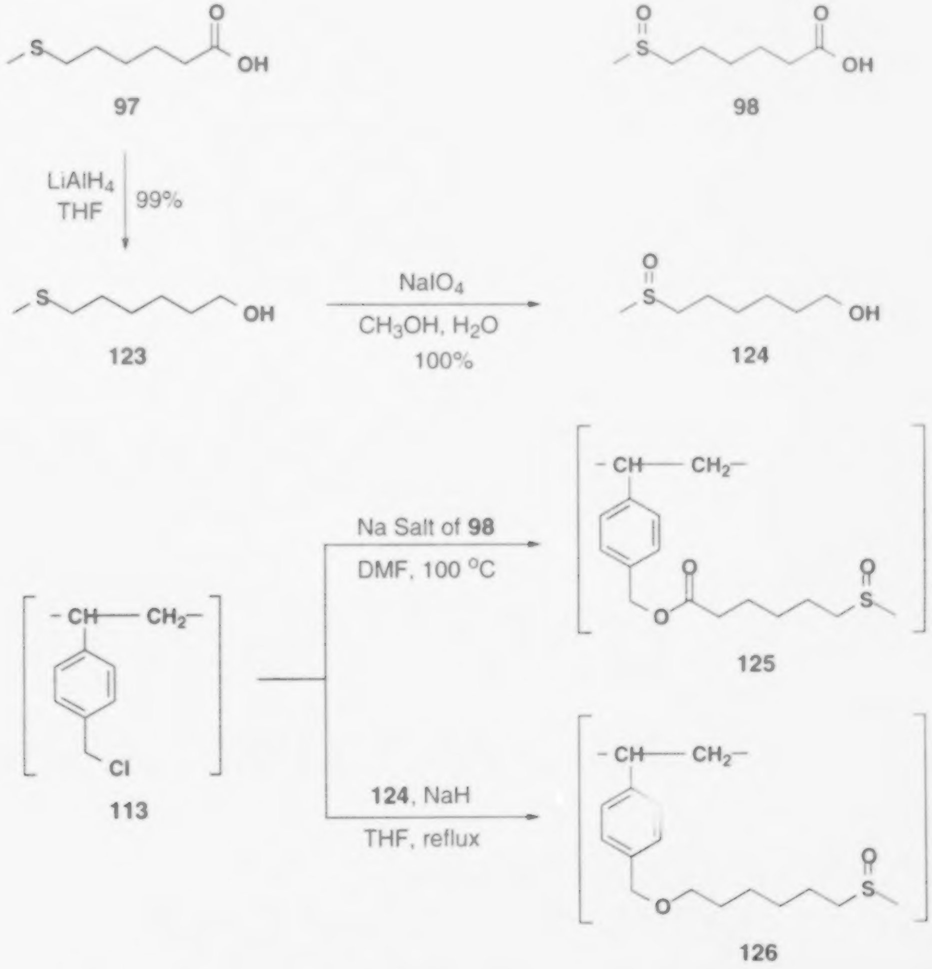
Although sodium metaperiodate is successful for the oxidation of sulfide **97** to give the corresponding sulfoxide, it does not oxidize polymer **118** under the same conditions, or at room temperature. On changing the solvent system to THF-H_2O (v/v 3:1), which is a much better solvent for the swelling of **118**, the periodate oxidation proceeds smoothly at 50 °C. No over-oxidation could be detected with excess oxidant or prolonged reaction time, and the sulfoxide polymer **119** obtained this way was much purer.

Polymer **119** works efficiently in the Swern oxidation (e.g. *endo*-borneol to camphor, 92% by ^{1}H NMR), but unfortunately, an obnoxious smell is produced during the reaction. Presumably, breakdown of the polymer after activation releases volatile sulfur-containing species (Scheme 45). The polymeric chlorosulfonium salt **121** may collapse to the benzyl cation **122** and methylsulfenyl chloride. This may react with water during the workup to form methanethiol, which can give the disulfide or other volatile molecules.



Scheme 45

To avoid this undesirable degradation process, a spacer can be placed between the sulfoxide functionality and polymeric aromatic ring. Compounds **98** and **124** were attached to the solid support through either an ester linkage[91] or an ether linkage,[87a] respectively (Scheme 46). Treatment of **113** with the sodium salt of **98** in DMF forms the resin **125** in 93% yield. Its IR spectrum shows strong absorptions at 1736 and 1029 cm^{-1}, corresponding to the carbonyl and sulfoxide functionalities. The ^{13}C NMR spectrum, which shows sharp signals for the side chain, as well as elemental analysis suggest complete substitution. Alcohol **124** is readily prepared by reduction of the carboxyl group of **97** with lithium aluminum hydride, and oxidation of the sulfur of the resultant compound **123** with sodium metaperiodate to the sulfoxide **124**. Treatment of **113** with the sodium alkoxide of **124** in THF gives the resin **126** in 95% yield based on elemental analysis of sulfur. Resin **126** does not show any sharp signals in its ^{13}C NMR spectrum, which suggests that side reactions may increase the degree of crosslinking in this polymer.


97
98
LiAlH4
THF
99%
123
NaIO4
CH3OH, H2O
100%
124
Na Salt of 98
DMF, 100 °C
125
113
124, NaH
THF, reflux
126

Scheme 46

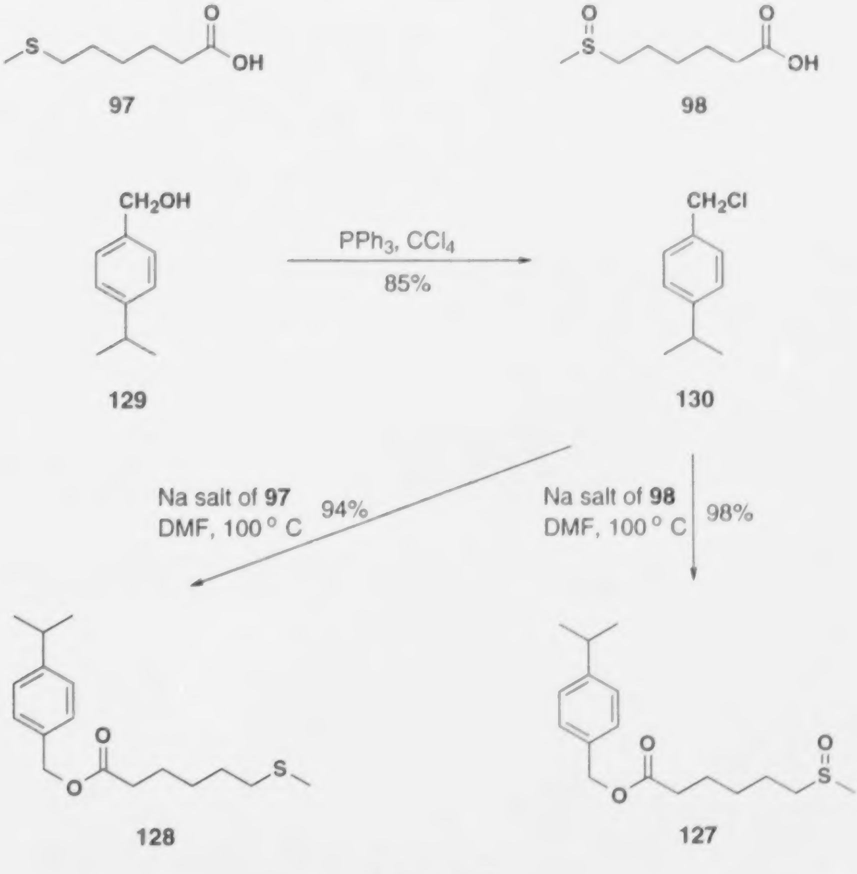
Exactly one equivalent of the polymeric reagent **125** is employed to oxidize *endo*-borneol; 92% of the alcohol is converted to camphor and 8% remains unchanged. This result indicates that 92% of the sulfur in **125** can be used for Swern oxidation. However, use of a two-fold excess of the reagent in the Swern process permits quantitative oxidation to camphor with no residual alcohol remaining, making it superior to dimsyl-derived reagent **114** and the soluble version using **98**. The fully reduced resin is quite sticky. Its IR spectrum displays a strong band for the carbonyl at 1744 cm^{-1} but no sulfoxide absorption. However, no sharp signals are visible in the ^{13}C NMR spectrum. The sulfoxide polymer was then regenerated by sodium metaperiodate oxidation, and examined for the oxidation of *endo*-borneol. This regenerated polymeric sulfoxide now allows only 50% of the sulfur to be used. Reagent **126** gives similar, but inferior, results to **125**. For comparison these results are summarized in Table 7.

Table 7 Regeneration of polymer reagents **125** and **126**

Cycle #	Polymer	Oxidation Capacity
1	**125**	92% of S (2.29 mmol/g)
2		50% of S (1.24 mmol/g)
1	**126**	72% of S (1.85 mmol/g)
2		45% of S (1.15 mmol/g)

Reagent **125**, with high sulfur loading and oxidation capacity, promises to maintain the efficiency of the classical Swern reaction and will enhance ease of product purification. However, its oxidation capacity decreases substantially when it is recycled. This may be attributed to the side reactions introduced by the Swern reaction or the structure of polystyrene. For example, the chlorosulfonium intermediate may alkylate the nearby aromatic rings in the polymer in Friedel-Crafts reaction, or sodium metaperiodate may oxidize at the benzylic position of the polymer backbone *via* a free radical mechanism. Both of these possibilities would result in crosslinking of the polymer.

In order to investigate these potential side reactions compounds **127** and **128**, in which the isopropyl group mimics the polymer chain, were designed for model studies (Scheme 47). Commercially available *p*-isopropylbenzyl alcohol **129** is converted to the corresponding chloride **130** in 85% yield by treatment with triphenylphosphine in carbon tetrachloride. Coupling of **130** with **98** in the same way as for the polymer reagent generates **127** in 98% yield. Compound **128** is available by similar coupling of **130** and **97**.


97
98
129
PPh3, CCl4
85%
130
Na salt of 97
DMF, 100 ° C
94%
Na salt of 98
DMF, 100 ° C
98%
128
127

Scheme 47

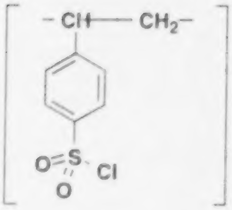
Sulfoxide **127** was used in slight excess (1.2 equiv) with oxalyl chloride (1.1 equiv) in the oxidation of *endo*-borneol; the alcohol is completely converted to camphor, and no products other than **128** and excess **127** are detectable. In order to closely mimic the microenvironment of polymer **125**, in which all the reactive ends are constantly surrounded by the styrene moieties, the reaction was repeated in toluene, and again did not show any side reactions. This indicates that the electrophilic chlorosulfonium species is unlikely to alkylate the aromatic rings. Sulfide **128** can be converted efficiently by sodium metaperiodate to the sulfoxide **127** in aqueous methanol at 0 °C, with no side reactions occurring under these conditions.

For the classical Swern procedure a typical recipe of reagents is as follows: DMSO (2.2 equiv), oxalyl chloride (1.2 equiv), alcohol (1 equiv) and triethylamine (3 equiv). When **127** (1 equiv) and excess of oxalyl chloride (1.3 equiv) are used for the oxidation of *endo*-borneol (1 equiv) a black solution is obtained. Although no side products were isolated in this case, the results suggest that oxalyl chloride may react with the polystyrene reagents. Our initial procedure had employed the polymer sulfoxide reagent **125** with excess of oxalyl chloride and alcohol to obtain fully reduced polymer for the subsequent regeneration. However, to avoid possible reactions of the polystyrene aromatic residues with oxalyl chloride, the polymer sulfoxide **125** was used in excess (1.5 equiv) with oxalyl chloride (1.1 equiv) and *endo*-borneol (1 equiv). The polymer recovered from this reaction gives a ^{13}C NMR spectrum with clear signals for both sulfide and sulfoxide side chains. Regeneration as before by sodium metaperiodate oxidation provides similar IR and ^{13}C NMR spectra to the original reagent **125**. Its oxidation capacity on *endo*-borneol was determined to be 78% of the total sulfur content. Results are summarized in Table 8. This improved procedure gives promise for both efficient oxidation of the alcohol substrate and reagent regeneration.

Table 8 Regeneration of polymer reagent **125** using the improved procedure

Cycle #	Polymer	Elemental Analysis			Oxidation Capacity
		C%	H%	S%	
1	Sulfoxide	68.38	7.34	7.95	92% of S (2.29 mmol/g)
	Sulfide	71.26	7.59	8.01	
2	Sulfoxide	65.55	7.24	8.22	78% of S (2.00 mmol/g)

The above results show that Merrifield resin backbone, although functional, may not be ideal for Swern oxidation due to the crosslinking problem with oxalyl chloride. Future work can examine other functionalized polymers such as chlorosulfonylpolystyrene (**131**) and polyethylene glycol monomethyl ether[92] (MeO-PEG) (**132**). The direct attachment of the sulfonyl group deactivates the aromatic ring, which may minimize side reactions on the styrene moieties (Figure 17). Polymer **131** is commercially available, but can also be easily prepared from Dowex resin (**133**) by treatment with thionyl chloride. Sulfoxide **98** could be linked to the polymer through a spacer to form the reagent **134**. Alternatively, **98** could be converted to its amine, then directly load onto the polymer to give reagent **135**. MeO-PEG-supported reagent **136** could be obtained by coupling sulfoxide **98** with the polymer. Since MeO-PEG is soluble in certain solvents (e.g. CH_2Cl_2), the sulfoxide **136** would be expected to behave similarly, and thus the reaction could occur homogeneously. Recovery of the polymer bound reagent could be achieved by precipitation with diethyl ether and filtration. Reagents **134**, **135** and **136** may thus be recyclable polymer-supported reagents for Swern oxidation. These reagents will be investigated in future work.



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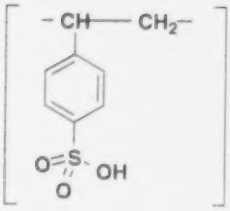

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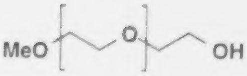

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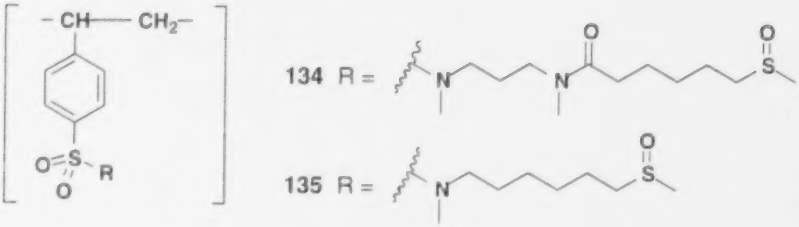
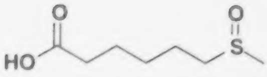


134 R =

135 R =



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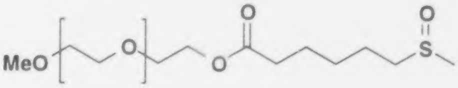


136

Figure 17

EXPERIMENTAL

General

All non-aqueous reactions requiring anhydrous conditions were performed under an atmosphere of dry argon using oven-dried glassware. All solvents for anhydrous reactions were dried according to Perrin *et al.*[93] THF, diethyl ether, benzene, and toluene were distilled over sodium and benzophenone under an argon atmosphere. Acetonitrile, dichloromethane, DMF, DMSO, triethylamine, pyridine, hexamethyldisilazane (HMDS) and *N,N*-diisopropylethylamine were distilled from calcium hydride. Anhydrous ethanol and methanol were purified by distillation from magnesium turnings and a catalytic amount of iodine. Water was obtained from a Milli-Q reagent water system (Millipore Corp.; Milford, MA). Solvents used for chromatography were distilled. Solvent evaporation was performed under reduced pressure below 40 °C using a Büchi rotary evaporator, followed by evacuation (<0.1 mm Hg) to constant sample weight. "Brine" refers to a saturated aqueous solution of NaCl. Unless otherwise specified, solutions of NH_4Cl, $NaHCO_3$, KOH and NaOH refer to aqueous solutions.

All reagents employed were of American Chemical Society (ACS) grade or finer, and were used without further purification unless otherwise mentioned. All commercially available labeled compounds were purchased from Cambridge Isotope Laboratories (Woburn, MA), with isotopic purity of 99%. Commercial organometallic reagents were obtained from Aldrich Chemical Co. *n*-Butyllithium solution was periodically titrated against menthol/phenanthroline. LHMDS was titrated against menthol/fluorene before use. Freeze-dried specimens of *Alternaria cinerariae* (ATCC 11784) were purchased from American Type Culture Collection (ATCC) (Rockville, Maryland). Cross-linked (divinyl benzene) chloromethylated polystyrene resin (4.15 mequiv/g, Bio-Beads S-X-1) was obtained from Bio-Rad Laboratories (Mississauga, ON).

Where possible all reactions were followed by thin layer chromatography (TLC) and visualized using UV fluorescence, iodine staining, phosphomolybdic acid/ceric sulfate/sulfuric acid (10 g : 1.25 g : 8% 250 mL) spray. Commercial thin layer and preparative layer chromatography plates were: normal silica (Merck 60 F-254) or reverse-phase (Merck RP-8 or RP-18 F-254S). Flash chromatography was performed according to Still *et al.*[94] using Merck type 60, 230-420 mesh silica gel. Reverse phase medium pressure liquid chromatography (MPLC) was performed on a Merck Lobar Lichroprep RP-8 column, Size B. All solvent mixtures are listed as volume ratios, and all medium pressure liquid chromatography was performed using solvents which were previously degassed under vacuum.

High performance liquid chromatography (HPLC) was performed on a Beckman System Gold instrument equipped with a model 166 variable wavelength UV detector set at 254 nm, and an Altex 210A injector with a 100 µL sample loop. The columns used were Waters Nova-Pak cartridges (reverse phase 8NVC18 4µ C_{18} column, 1 x 10 cm) encased in a Waters Z-module compression unit. HPLC grade acetonitrile (190 nm cutoff) and methanol were obtained from Terochem (Edmonton, AB). All HPLC solvents were prepared fresh daily and filtered with a Millipore filtration system under vacuum before use.

Melting points were determined on a Thomas-Hoover or Büchi oil immersion apparatus using open capillary tubes and are uncorrected. Optical rotations were measured on a Perkin Elmer 241 polarimeter with a microcell (10.0 cm path length, 0.9 mL) at ambient temperature. All specific rotations reported were measured at the sodium D line and were referenced against air. Infrared (IR) were recorded on a Nicolet 7199 or 20SX FT-IR spectrometer. Cast refers to the evaporation of a solution on a NaCl plate. Mass spectra (MS) were recorded on Kratos AEI MS-50 (high resolution, electron impact ionization (EI)), MS-12 (chemical ionization (CI), NH_3), and MS-9 (fast atom bombardment (FAB), argon) instruments. Cleland matrix used in FAB refers to a 5:1 mixture of dithiothreitol and dithioerythritol. Microanalyses were obtained on Perkin Elmer 240 or Carlo Erba 1180 elemental analyzers.

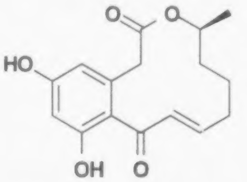
Nuclear magnetic resonance (NMR) spectra were obtained on Bruker WH-200, AM-300, WM-360, WH-400 instruments. ^{1}H NMR chemical shifts are reported in parts per million (ppm) downfield relative to tetramethylsilane (TMS) using the residual proton resonance of solvents as the reference: $CDCl_3$ δ 7.25, CD_2Cl_2 δ 5.32, D_2O δ 4.72, CD_3OD δ 3.30 (CD_3), $(CD_3)_2SO$ δ 2.49, $(CD_3)_2CO$ δ 2.04, CD_3CN δ 1.93. ^{13}C NMR shifts are reported relative to $CDCl_3$ δ 77.0, CD_2Cl_2 δ 53.8, CD_3OD δ 49.0, $(CD_3)_2SO$ δ 39.5, $(CD_3)_2CO$ δ 29.8 (CD_3), CD_3CN δ 117.7 (CN). Selective homonuclear decoupling, attached proton test (APT) were occasionally used for signal assignments.[95] ^{1}H NMR data are tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet), number of protons, coupling constant(s) in Hertz (Hz), and assignment.

All glassware employed in reactions and handling of resin was pretreated with a 10% solution of Surfasil siliconizing agent (Pierce) in hexane and oven dried at 140 °C in order to minimize loss of resin due to adhesion. Gentle stirring (50-140 rpm) was used at all times to avoid mechanical destruction of resin particles. The term "washing" implies the suspension of the resin in solvent, stirring 15-20 min followed by removal of solvent by suction filtration. For ^{13}C NMR analysis the resin was first swollen in the appropriate deuterated solvent, then analyzed in the conventional manner.

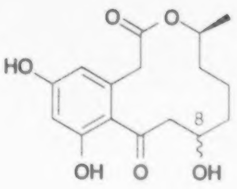
β-Oxidation inhibitors **25** and **26**, prepared by Dr. Zhe Li and Dr. Fionna M. Martin in our group, were used for feeding experiments. *N*-Acetylcysteamine (**11**) and (carbomethoxymethylene)triphenylphosphorane (labeled or unlabeled) was synthesized using Dr. Zhe Li's procedure.[27]

Fermentation of *Alternaria cinerariae* and Isolation of Dehydrocurvularin and 8-Hydroxycurvularin The cultures of *Alternaria cinerariae* (ATCC 11784) were maintained on slants made from potato dextrose agar (Difco, 39 g/L). Potato dextrose broth (Difco, 30 g) was dissolved in Milli-Q water (1.25 L), and equally divided into ten

500 mL Erlenmeyer flasks. After autoclaving and cooling to room temperature, the flasks were inoculated with spore suspensions from 2 slants, then placed on a rotary fermenter and incubated at 26 °C and 165 rpm for 7 days in the dark. The cultures were filtered and washed with EtOAc (500 mL), the filtrate was extracted with EtOAc (3 x 800 mL). Concentration after drying (Na_2SO_4) provided a yellow gum (343 mg). Flash chromatography on SiO_2 gave dehydrocurvularin (217 mg) and 8-hydroxycurvularin (79 mg) with spectroscopic data as shown below. To obtain the production curves another ten flasks were fermented. One flask was worked up each day after day three. The crude extracts were individually analyzed with HPLC, using standards for quantitative calibration.



Dehydrocurvularin (1) Mp 228 °C (dec.) (lit.[19] 223-224 °C); IR (KBr) 3424 (br s), 3308 (br s), 1723 (s), 1595 (s), 1154 (m), 842 (m) cm^{-1}; ^{1}H NMR (300 MHz, acetone-d_6) δ 12.35 (br s, 1 H, Ar-O$\underline{\text{H}}$), 9.21 (br s, 1 H, Ar-O$\underline{\text{H}}$), 6.77 (d, 1 H, *J* = 15.4 Hz, H-9), 6.57 (ddd, 1 H, *J* = 15.4, 8.6, 4.9 Hz, H-8), 6.34 (d, 1 H, *J* = 2.4 Hz, Ar-$\underline{\text{H}}$), 6.29 (d, 1 H, *J* = 2.4 Hz, Ar-$\underline{\text{H}}$), 4.71 (m, 1 H, H-4), 4.07 (d, 1 H, *J* = 17.7 Hz, 1 x H-1), 3.60 (d, 1 H, *J* = 17.7 Hz, 1 x H-1), 2.48-2.22 (m, 2 H, 2 x H-7), 2.10-1.53 (m, 4 H), 1.17 (d, 3 H, *J* = 6.5 Hz, C$\underline{\text{H}}_3$); ^{13}C NMR (50 MHz, acetone-d_6) δ 197.24 (C-10), 171.76 (C-2), 165.85 (C-11), 162.94 (C-13), 149.58 (C-8), 139.33 (C-15), 132.56 (C-9), 115.72 (C-16), 113.63 (C-14), 102.91 (C-12), 72.85 (C-4), 43.62 (C-1), 34.81(C-5), 33.11 (C-7), 24.90 (C-6), 20.26 ($\underline{\text{C}}$$H_3$); MS (EI) *m/z* (relative intensity) 290 (M^+, 100), 203 (49), 190 (17), 177 (22).

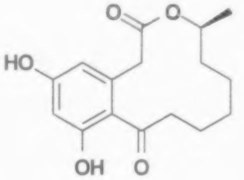


8-Hydroxycurvularin (3) A mixture of two isomers (1:2.6 ratio, determined by ^{1}H NMR) . Mp 115-117 °C (lit.[14b] 150-152 °C for α-isomer, 138-140 °c for β-isomer); IR (KBr) 3414 (br s), 2931 (m), 1703 (s), 1611 (s), 1267 (s), 1160 (m), 842 (m) cm^{-1}; ^{1}H NMR (300 MHz, acetone-d_6) δ 9.05 (br s, 1 H, Ar-OH), 8.75 (br s, 1 H, Ar-OH), 6.40 (d, 1 H, *J* = 2.3 Hz, Ar-H), 6.34 (d, 1 H, *J* = 2.3 Hz, Ar-H), 4.82 (m, 1 H, H-4), 4.10 (m, 1 H, H-8), 3.85-2.75 (m, 4 H, 2 x H-1, 2 x H-9), 1.80-1.20 (m, 6 H, 2 x H-5, 2 x H-6, 2 x H-7), 1.10 (d, 3 H, *J* = 6.2 Hz, CH$_3$); ^{13}C NMR (50 MHz, acetone-d_6) for the major isomer δ 204.47 (C-10), 170.50 (C-2), 160.02 (C-11), 158.06 (C-13), 136.55 (C-15), 120.74 (C-16), 111.72 (C-14), 102.32 (C-12), 73.03 (C-4), 66.82 (C-8), 53.96 (C-9), 39.09 (C-1), 35.16 (C-5), 32.13 (C-7), 22.12 (C-6), 21.02 (CH$_3$); for the minor isomer δ 204.06 (C-10), 170.32 (C-2), 160.16 (C-11), 158.74 (C-13), 137.07 (C-15), 120.22 (C-16), 112.10 (C-14), 102.32 (C-12), 71.21 (C-4), 67.57 (C-8), 53.27 (C-9), 39.55 (C-1), 35.74 (C-5), 31.61 (C-7), 22.12 (C-6), 21.02 (CH$_3$); HRMS (EI) calcd for $C_{16}H_{20}O_6$ 308.1260, found 308.1261 (M$^+$, 5); MS(FAB, Cleland) *m/z* (relative intensity) 309 (MH$^+$, 85), 291 (39), 195 (100).

Fermentation of *A. cinerariae* to Incorporate Advanced Precursors The same conditions as described above were used except that after 96 h, the mycelia from two flasks were filtered and washed with replacement media consisting of: glucose (100 g); Na_2HPO_4 (1 g); $MgSO_4 \cdot 7H_2O$ (0.5 g); KCl (0.5 g); $FeSO_4 \cdot 7H_2O$ (0.01 g) per litre. The washed mycelia were further incubated in a flask containing 125 mL of replacement media. Labeled precursor in 98% EtOH (1 mL) and β-oxidation inhibitor (35 mg) in 98%

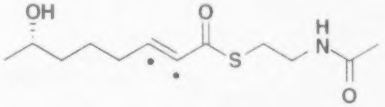
EtOH (1 mL) were then added in six equal aliquots at 12 h intervals. The culture was extracted 12 h after the last feeding.

Fermentation of *A. cinerariae* in Stationary Culture and Isolation of Curvularin Potato dextrose broth (Difco, 19.2 g) was dissolved in Milli-Q water (800 mL) in a Fernbach flask (2.8 L), and autoclaved. The medium was inoculated with spore suspensions from 2 slants, then placed in an incubator at 28 °C for 20 days in the dark. The cultures were filtered and washed with EtOAc (400 mL), the mycelia were homogenized and washed twice with EtOAc. The combined filtrates were extracted with EtOAc (3 x 500 mL). Concentration after drying (Na_2SO_4) provided a yellow gum (195 mg). Flash chromatography on SiO_2 (20:1 $CHCl_3$-CH_3OH) gave dehydrocurvularin (53 mg) and curvularin (6 mg) and a trace amount of 8-hydroxycurvularin.

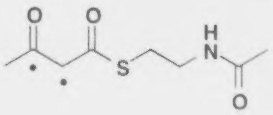


Curvularin (2) The procedure of Arai *et al.*[19] was used. A solution of **1** (150 mg, 0.52 mmol) and PtO_2 catalyst (5 mg) in EtOAc (50 mL) was stirred under H_2 atmosphere for 2 h. After filtration and evaporation of solvent the crude product was purified by flash chromatography (25:1 $CHCl_3$-CH_3OH) to give curvularin (**2**) (143 mg, 95%). Mp 200-202 °C (lit.[19] 202 °C); IR (KBr) 3309 (br s), 2931 (m), 1701 (s), 1608 (s), 1466 (s), 1327 (s), 1160 (s), 844 (m) cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 9.16 (s, 1 H, Ar-OH), 8.76 (s, 1 H, Ar-OH), 6.37 (d, 1 H, *J* = 2.3 Hz, Ar-H), 6.33 (d, 1 H, *J* = 2.3 Hz, Ar-H), 4.90 (m, 1 H, H-4), 3.79-3.66 (AB, 2 H, 2 x H-1), 3.14-3.03 (m, 1 H, 1 x H-9), 2.80-2.70 (m, 1 H, 1 x H-9), 1.79-1.67 (m, 2 H, 2 x H-5), 1.63-1.20 (m, 6 H, 2 x H-6, 2 x H-7, 2 x H-8), 1.10 (d, 3 H, *J* = 6.3 Hz, $C\underline{H}_3$); ^{13}C NMR (50 MHz, acetone-d_6) δ 206.72 (C-10), 171.02

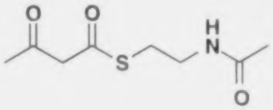
(C-2), 160.18 (C-11), 158.33 (C-13), 136.94 (C-15), 121.40 (C-16), 112.30 (C-14), 102.65 (C-12), 72.59 (C-4), 44.00 (C-1), 39.75 (C-9), 32.96 (C-5), 27.57 (C-8), 24.54 (C-6), 23.53 (C-7), 20.53 ($\underline{\mathrm{C}}\mathrm{H}_3$); MS (EI) *m/z* (relative intensity) 292 (M$^+$, 45), 205 (3), 195 (100), 167 (94), 150 (65).



NAC (*S*)-[2,3-$^{13}\mathrm{C}_2$]-7-hydroxyoct-2-enoate (5) The procedure for the conversion of **23a** to **8a** was adopted. Thus, silyl ether **43** (121 mg, 0.322 mmol) and boron trifluoride diethyl etherate (0.50 mL, 4.0 mmol) in $\mathrm{CH_2Cl_2}$ (20 mL) gave alcohol **5** (63.5 mg, 75%). IR ($\mathrm{CHCl_3}$ cast) 3288 (br s), 2931 (m), 1658 (s), 1578 (s), 1552 (s) cm^{-1}; ^{1}H NMR (300 MHz, $\mathrm{CDCl_3}$) δ 6.90 (ddt, 1 H, *J* = 153.2, 15.5, 7.2 Hz, $^{13}\mathrm{C}\underline{\mathrm{H}}\mathrm{=}^{13}\mathrm{CHCO}$), 6.26 (br s, 1 H, $\mathrm{N}\underline{\mathrm{H}}$), 6.11 (dd, 1 H, *J* = 160.9, 15.5 Hz, $^{13}\mathrm{C}\underline{\mathrm{H}}\mathrm{CO}$), 3.78 (m, 1 H, $\mathrm{C}\underline{\mathrm{H}}\mathrm{(OH)}$), 3.42 (q, 2 H, *J* = 6.1 Hz, $\mathrm{C}\underline{\mathrm{H}}_2\mathrm{NH}$), 3.06 (t, 2 H, *J* = 6.4 Hz, $\mathrm{SC}\underline{\mathrm{H}}_2$), 2.20 (m, 3 H, $\mathrm{C}\underline{\mathrm{H}}_2{}^{13}\mathrm{CH} + \mathrm{O}\underline{\mathrm{H}}$), 1.95 (s, 3 H, $\mathrm{C}\underline{\mathrm{H}}_3\mathrm{CO}$), 1.70-1.35 (m, 4 H, $\mathrm{CH(OH)C}\underline{\mathrm{H}}_2\mathrm{C}\underline{\mathrm{H}}_2$), 1.17 (d, 3 H, *J* = 6.2 Hz, $\mathrm{C}\underline{\mathrm{H}}_3\mathrm{CH(OH)}$); ^{13}C NMR (75 MHz, $\mathrm{CDCl_3}$) δ 190.23 (d, *J* = 61.0 Hz, $\underline{\mathrm{C}}\mathrm{OS}$), 170.43 ($\underline{\mathrm{C}}\mathrm{OCH_3}$), 146.13 (d, *J* = 69.4 Hz, enriched, $^{13}\underline{\mathrm{C}}\mathrm{H=}^{13}\mathrm{CHCO}$), 128.41 (d, *J* = 69.4 Hz, enriched, $^{13}\underline{\mathrm{C}}\mathrm{HCO}$), 67.50 ($\underline{\mathrm{C}}\mathrm{H(OH)}$), 39.65 ($\underline{\mathrm{C}}\mathrm{H_2NH}$), 38.46 ($\mathrm{CH(OH)}\underline{\mathrm{C}}\mathrm{H_2}$), 32.03 (d, *J* = 41.1 Hz, $\mathrm{CH_2}{}^{13}\mathrm{CH}$), 28.16 ($\mathrm{S}\underline{\mathrm{C}}\mathrm{H_2}$), 24.05 ($\underline{\mathrm{C}}\mathrm{H_2CH_2}{}^{13}\mathrm{CH}$), 23.53 ($\underline{\mathrm{C}}\mathrm{H_3CH(OH)}$), 23.07 ($\underline{\mathrm{C}}\mathrm{H_3CO}$); MS (CI, $\mathrm{NH_3}$) 279 ($\mathrm{MNH_4^+}$, 29), 262 (MH$^+$, 76); HRMS (EI) *m/z* (relative intensity) calcd for $^{13}\mathrm{C_2C_{10}H_{21}NO_3S}$ 261.1309, found 261.1297 (M$^+$, 1), 143.0980 (39), 119.0404 (85).



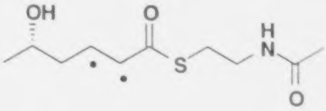
NAC [2,3-$^{13}C_2$]Acetoacetate (7) The alternative procedure as described above for unlabeled compound was used. The product was further purified using MPLC (Merck, RP-8, 40-63 μm), eluting with 95:5 H_2O-CH_3CN, to give pure **7** (42% yield from **14**). IR ($CHCl_3$ cast) 3287 (br), 1667 (s), 1547 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), for keto form δ 6.21 (br s, 1 H, N$\underline{H}$), 3.69 (dd, 2 H, J = 131.0, 6.2 Hz, $^{13}CO^{13}C\underline{H}_2$), 3.43 (q, 2 H, J = 6.1 Hz, $C\underline{H}_2NH$), 3.08 (t, 2 H, J = 6.4 Hz, $SC\underline{H}_2$), 2.25 (dd, 3 H, J = 6.2, 1.3 Hz, $C\underline{H}_3{}^{13}CO$), 1.96 (s, 3 H, $COC\underline{H}_3$); for enol form δ 12.55 (s, 1 H, $O\underline{H}$), 6.21 (br s, 1 H, $N\underline{H}$), 5.45 (dd, 1 H, J = 167.3, 4.4 Hz, $^{13}C{=}^{13}C\underline{H}$), 3.43 (q, 2 H, J = 6.1 Hz, $C\underline{H}_2NH$), 3.04 (t, 2 H, J = 6.3 Hz, $SC\underline{H}_2$), 1.96 (s, 3 H, $COC\underline{H}_3$), 1.93 (dd, 3 H, J = 6.6, 4.2 Hz, $C\underline{H}_3{}^{13}C$); ^{13}C NMR (100 MHz, $CDCl_3$), for keto form δ 199.83 (d, J = 36.3 Hz, enriched, $^{13}\underline{C}O$), 192.12 ($\underline{C}OS$), 170.51 ($\underline{C}OCH_3$), 57.93 (d, J = 36.3 Hz, enriched, $^{13}\underline{C}H_2COO$), 39.05 ($\underline{C}H_2NH$), 30.54 ($\underline{C}H_3{}^{13}CO$), 29.10 ($SC\underline{H}_2$), 23.02 ($CO\underline{C}H_3$); for enol form δ 194.11 ($\underline{C}OS$), 173.91 (d, J = 69.9 Hz, enriched, $^{13}\underline{C}{=}^{13}CH$), 170.38 ($\underline{C}OCH_3$), 99.78 (d, J = 69.9 Hz, enriched, $^{13}C{=}^{13}\underline{C}H$), 39.76 ($C\underline{H}_2NH$), 27.66 ($S\underline{C}H_2$), 23.08 ($CO\underline{C}H_3$), 20.90 ($\underline{C}H_3{}^{13}C$); HRMS (EI) calcd for $^{13}C_2C_6H_{13}NO_3S$ 205.0683, found 205.0680; MS (CI, NH_3) *m/z* (relative intensity) 223 (MNH_4^+, 71), 206 (MH^+, 21).



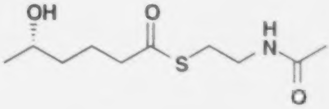
NAC Acetoacetate (7a) A modification of the method of Oikawa *et al.*[50a] was used. Compound **10** (1.00 g, 5.38 mmol) and *N*-acetylcysteamine (1.28 g, 1.07 mmol) were mixed in benzene (30 mL). The mixture was heated at reflux, and the reaction was monitored with tlc until all **10** had been consumed. The crude product was found to be a mixture of **7a** and excess *N*-acetylcysteamine which was difficult to separate with SiO_2

chromatography. Excess **10** (1.80 g, 8.71 mmol) was then added to the mixture which was then heated at reflux for a further 2 h. After removal of the solvent the reddish brown residue was purified by SiO_2 column chromatography (5% CH_3OH in $CHCl_3$) to give pure **7a** as a solid (1.40 g, 64%). IR ($CHCl_3$ cast) 3287 (br), 1722 (m), 1674 (s), 1547 (m) cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) a mixture of keto and enol, (69:31), for keto form, δ 6.32 (br s, 1 H, $N\underline{H}$), 3.69 (s, 2 H, $COC\underline{H}_2CO$), 3.44 (q, 2 H, *J* = 6.0 Hz, $C\underline{H}_2NH$), 3.08 (t, 2 H, *J* = 6.3 Hz, $SC\underline{H}_2$), 2.25 (s, 3 H, $C\underline{H}_3CO$), 1.98 (s, 3 H, $NHCOC\underline{H}_3$); for enol form, δ 12.55 (s, 1 H, $O\underline{H}$), 6.31 (br s, 1 H, $N\underline{H}$), 5.45 (s, 1 H, $C\underline{H}=C$), 3.44 (q, 2 H, *J* = 6.0 Hz, $C\underline{H}_2NH$), 3.06 (t, 2H, *J* = 6.2 Hz, $SC\underline{H}_2$), 1.98 (s, 3 H, $NHCOC\underline{H}_3$), 1.96 (s, 3 H, $C\underline{H}_3C(OH)$); ^{13}C NMR (50 MHz, $CDCl_3$), for keto form δ 199.85 ($\underline{C}O$), 192.06 ($\underline{C}OS$), 171.19 ($\underline{C}OCH_3$), 57.91 ($CO\underline{C}H_2CO$), 39.24 ($\underline{C}H_2NH$), 30.34 ($\underline{C}H_3CO$), 28.90 ($S\underline{C}H_2$), 22.69 ($NHCO\underline{C}H_3$); for enol form δ 194.01 ($\underline{C}OS$), 174.00 ($\underline{C}(OH)=CH$), 171.09 ($\underline{C}OCH_3$), 99.79 ($C(OH)=\underline{C}H$), 39.97 ($C\underline{H}_2NH$), 27.47 ($S\underline{C}H_2$), 22.69 ($\underline{C}H_3CO$), 20.95 ($\underline{C}H_3C(OH)$); HRMS (EI) *m/z* (relative intensity) calcd for $C_8H_{13}NO_3S$ 203.0616, found 203.0611 (M^+, 6), 119.0403 (82).

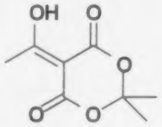
An Alternative Procedure for Preparation of 7a Ethyl acetoacetate was hydrolyzed following Hall's procedure[96] to give the lithium acetoacetate in 51% yield. The lithium salt (216 mg, 2.00 mmol) was dissolved in a minimum amount of H_2O. The solution was then cooled in an ice bath, and acidified with cold dilute H_2SO_4 to pH 1. After saturation with NaCl the solution was extracted with ether (4 x 10 mL), and dried (Na_2SO_4). After concentration *in vacuo* the colorless oil (194 mg) was dissolved in CH_2Cl_2 (20 mL) at 0 °C. Molecular sieves (5 g) were added and stirred for 15 min. Solutions of DCC (454 mg, 2.20 mmol) and DMAP (10 mg) in CH_2Cl_2 (5 mL), and *N*-acetylcysteamine (262 mg, 2.20 mmol) in CH_2Cl_2 (5 mL) were added simultaneously *via* two syringes. The mixture was then stirred at room temperature overnight. After filtration and evaporation of solvent the residue was purified by SiO_2 column chromatography (5% CH_3OH in $CHCl_3$) to afford the product (281 mg) as a mixture of **7a** and *N*-acetylcysteamine (ca. 1 : 1 ratio, determined by ^{1}H NMR).



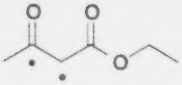
NAC (*S*)-[2,3-$^{13}C_2$]-5-Hydroxyhexanoate (8) The same procedure as for unlabeled compound **8a** was used. IR ($CHCl_3$ cast) 3293 (br s), 2929 (s), 1686 (m), 1686 (s), 1656 (s), 1554 (m), 1021 (m) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.25 (br s, 1 H, N$\underline{H}$), 3.77 (m, 1 H, C$\underline{H}$(OH)), 3.40 (q, 2 H, *J* = 6.2 Hz, C$\underline{H}_2$NH), 3.00 (t, 2 H, *J* = 6.4 Hz, SC$\underline{H}_2$), 2.58 (dm, 2 H, *J* = 128.7 Hz, ^{13}C$\underline{H}_2$CO), 2.39 (br s, 1 H, O$\underline{H}$), 1.95 (m, 3 H, COC$\underline{H}_3$), 1.72 (dm, 2 H, *J* = 132.6 Hz, ^{13}C$\underline{H}_2$^{13}CH$_2$CO), 1.45 (m, 2 H, CH(OH)C$\underline{H}_2$), 1.17 (d, 3 H, *J* = 6.2 Hz, C$\underline{H}_3$CH(OH)); ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.82 (d, *J* = 47.2 Hz, $\underline{C}$OS), 170.32 ($\underline{C}$OCH$_3$), 67.25 ($\underline{C}$H(OH)), 43.78 (d, *J* = 33.9 Hz, enriched, $^{13}\underline{C}$H$_2$COS), 39.47 ($\underline{C}$H$_2$NH), 38.47 (d, *J* = 35.1 Hz, CH(OH)$\underline{C}$H$_2$), 28.43 ($\underline{C}$H$_2$S), 23.44 ($\underline{C}$H$_3$CH(OH)), 23.06 ($\underline{C}$H$_3$CO), 21.74 (enriched, d, *J* = 33.9 Hz, $^{13}\underline{C}$H$_2$^{13}CH$_2$CO); MS (CI, NH_3) *m/z* (relative intensity) 253 (MNH$_4^+$, 28), 236 (MH$^+$, 47); HRMS (EI) *m/z* (relative intensity) 119.0396 (60), 117.0825 (18).



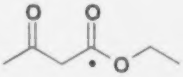
NAC (*S*)-5-Hydroxyhexanoate (8a) To a solution of silyl ether **23a** (35.4 mg, 0.102 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added boron trifluoride diethyl etherate (0.30 mL, 2.4 mmol). After stirring at 0 °C for 2 h the solution was neutralized with 5% Na_2CO_3, extracted with $CHCl_3$ (4 x 25 mL), and the combined extracts were dried (Na_2SO_4). Removal of solvent gave alcohol **8a** (23.2 mg, 98%). Analyses were done directly without further purification. IR ($CHCl_3$ cast) 3290 (br m), 2927 (s), 1690 (s), 1657 (s), 1552 (m) cm^{-1}; ^{1}H NMR (300 MHz, $\mathrm{CDCl_3}$) δ 6.01 (br s, 1 H, $\mathrm{N\underline{H}}$), 3.80 (m, 1 H, $\mathrm{C\underline{H}(OH))}$, 3.43 (q, 2 H, $J = 6.2$ Hz, $\mathrm{C\underline{H}_2NH}$), 3.02 (t, 2 H, $J = 6.4$ Hz, $\mathrm{SC\underline{H}_2}$), 2.61 (t, 2 H, $J = 7.3$ Hz, $\mathrm{C\underline{H}_2CO}$), 1.97 (m, 3 H, $\mathrm{COC\underline{H}_3}$), 1.90-1.60 (m, 2 H, $\mathrm{C\underline{H}_2CH_2CO}$), 1.47 (m, 2 H, $\mathrm{CH(OH)C\underline{H}_2}$), 1.19 (d, 3 H, $J = 6.2$ Hz, $\mathrm{C\underline{H}_3CH(OH))}$; ^{13}C NMR (75 MHz, $\mathrm{CDCl_3}$) δ 199.97 ($\mathrm{\underline{C}OS}$), 170.34 ($\mathrm{\underline{C}OCH_3}$), 67.40 ($\mathrm{\underline{C}H(OH)}$), 43.82 ($\mathrm{\underline{C}H_2CO}$), 39.58 ($\mathrm{\underline{C}H_2NH}$), 38.52 ($\mathrm{CH(OH)\underline{C}H_2}$), 28.38 ($\mathrm{S\underline{C}H_2}$), 23.52 ($\mathrm{\underline{C}H_3CH(OH)}$), 23.14 ($\mathrm{CO\underline{C}H_3}$), 21.78 ($\mathrm{\underline{C}H_2CH_2CO}$); MS (CI, $\mathrm{NH_3}$) *m/z* (relative intensity) 251 ($\mathrm{MNH_4^+}$, 56), 234 ($\mathrm{MH^+}$, 61); HRMS (EI) *m/z* (relative intensity) 119.0403 (62), 115.0757 (18).



5-Acetyl-2,2-dimethyl-1,3-dioxane-4,6-one (**10**) The procedure of Oikawa *et al.*[50a] was followed. Pyridine (4.63 mL, 57.2 mmol) was added to a solution of Meldrum's acid (3.30 g, 22.9 mmol) in $\mathrm{CH_2Cl_2}$ (20 mL) at 0 °C. To the resulting colorless solution acetyl chloride (1.63 mL, 22.9 mmol) in $\mathrm{CH_2Cl_2}$ (10 ml) was added over a period of 20 min. The mixture was stirred at 0 °C for 45 min and then at room temperature for 45 min. The dark orange solution was poured into a mixture of 1N HCl and crushed ice, and extracted with $\mathrm{CH_2Cl_2}$ (3 x 100 mL). The combined organic layers were washed once with 1 N HCl and once with brine, and dried ($\mathrm{Na_2SO_4}$). After evaporation of solvent the yellow solid was recrystallized with $\mathrm{Et_2O}$-hexane to afford **10** (3.10 g, 73%), which exists exclusively in its enol form. IR ($\mathrm{CHCl_3}$ cast) 1742 (m), 1668 (m), 1557 (s), 1207 (m) cm^{-1}; ^{1}H NMR (360 MHz, $\mathrm{CDCl_3}$) δ 11.70 (s, 1 H, $\mathrm{O\underline{H}}$), 2.67 (s, 3 H, $\mathrm{C\underline{H}_3C(OH)}$), 1.73 (s, 6 H, $\mathrm{(C\underline{H}_3)_2C}$); ^{13}C NMR (75 MHz, $\mathrm{CDCl_3}$) δ 194.60 ($\mathrm{CH_3\underline{C}(OH)}$), 170.19 and 160.44 (2 x $\mathrm{\underline{C}O}$), 104.89 ($\mathrm{\underline{C}(CO)_2}$), 91.83 ($\mathrm{\underline{C}(CH_3)_2}$), 26.83 ($\mathrm{C(\underline{C}H_3)_2}$), 23.47 ($\mathrm{\underline{C}H_3C(OH)}$); MS (EI) *m/z* (relative intensity) calcd for $\mathrm{C_8H_{10}O_5}$ 186.0528, found 186.0528 ($\mathrm{M^+}$, 16), 171.0300 (6), 129.0188 (50); Anal. Calcd for $\mathrm{C_8H_{10}O_5}$: C, 51.61; H, 5.41. Found: C, 51.66; H, 5.23.

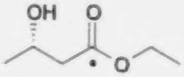


Ethyl [2,3-$^{13}C_2$]Acetoacetate (14) A modification of the procedure of Cane and Block[97] was used. LiHMDS was prepared by the addition of *n*-BuLi (2.5 M in hexane, 20.5 mL, 51 mmol) to HMDS (10.8 mL, 51.2 mmol) in THF (20 mL) at -78 °C. After the mixture had been warmed to room temperature, the solvent and unreacted HMDS were then removed *in vacuo*, and further dried under high vacuum. The white solid lithium salt was redissolved in THF (30 mL) at -78 °C. Ethyl [2-^{13}C] acetate (2.05 g, 23.0 mmol) was added and the solution stirred for 15 min at -78 °C. [1-^{13}C]Acetyl chloride (1.83g, 23.0 mmol) was introduced dropwise, and the mixture was stirred for 1 h at -78 °C. After warming to 20 °C, the reaction mixture was quenched by addition of 2N HCl (100 mL), and the solution was extracted with ether (3 x 100 mL). The combined organic layers were washed with brine, and dried (Na_2SO_4). After concentration *in vacuo* the resulting yellow residue was distilled to afford **14** (2.13 g, 70%). Bp 75-78 °C (12 mm Hg); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.20 (q, 2 H, $OC\underline{H}_2$), 3.45 (dd, 2 H, *J* = 130.2, 6.2 Hz, $^{13}CO^{13}C\underline{H}_2$), 2.27 (dd, 3 H, *J* = 7.4, 1.4 Hz, $C\underline{H}_3{}^{13}CO$), 1.29 (t, 3 H, *J* = 7.2 Hz, $CH_2C\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.54 (d, *J* = 38.5 Hz, enriched, $CH_3{}^{13}\underline{C}O$), 167.07 ($\underline{C}O$), 61.36 ($O\underline{C}H_2$), 50.12 (d, *J* = 37.7 Hz, enriched, $^{13}\underline{C}H_2CO$), 26.79 ($\underline{C}H_3{}^{13}CO$), 14.06 ($CH_2\underline{C}H_3$); HRMS (EI) *m/z* (relative intensity) calcd for $^{13}C_2C_4H_{10}O_3$ 132.0697, found 132.0712 (M^+, 13), 87.0354 (24).



Ethyl [1-^{13}C]Acetoacetate (14a) The same procedure as for **14** was used. IR ($CHCl_3$ cast) 2985 (m), 1729 (s), 1691 (s), 1146 (s) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.16 (dq, 2 H, *J* = 3.2, 7.1 Hz, $OC\underline{H}_2CH_3$), 3.41 (d, 2 H, *J* = 7.4 Hz, $COC\underline{H}_2{}^{13}CO$), 2.23 (s, 3 H,

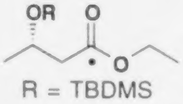
$\mathrm{C\underline{H}_3CO}$), 1.24 (t, 3 H, J = 7.1 Hz, $\mathrm{OCH_2C\underline{H}_3}$); ^{13}C NMR (100 MHz, $\mathrm{CDCl_3}$) δ 200.45 ($\mathrm{CH_3\underline{C}O}$), 166.98 (enriched, $\mathrm{^{13}\underline{C}O}$), 61.19 ($\mathrm{O\underline{C}H_2CH_3}$), 49.97 (d, J = 58.1 Hz, $\mathrm{\underline{C}H_2{}^{13}CO}$), 29.94 ($\mathrm{\underline{C}H_3CO}$), 13.93 ($\mathrm{OCH_2\underline{C}H_3}$); HRMS (EI) *m/z* (relative intensity) calcd for $\mathrm{^{13}CC_5H_{10}O_3}$ 131.0664, found 131.0664 ($\mathrm{M^+}$, 50).



Ethyl (*S*)-[1-^{13}C]-3-hydroxybutanoate (16) Dr. Zhe Li's procedure[27] was followed. D Glucose (70 g) and baker's yeast (Fleischmann's, 64 g) were dissolved in 0.1 M potassium phosphate buffer (1 L, pH 7.00). After the mixture was stirred at 33 °C for 30 min, **14a** (6.50 g, 49.6 mmol) in 98% ethanol (15 mL) was added. The mixture was stirred at 33 °C for ca. 7h until the extract showed a negative test on $\mathrm{FeCl_3/CHCl_3}$. It was then filtered through a pad of Celite 545, and the filtrate was extracted with $\mathrm{Et_2O}$ (4 x 400 mL). The dried ($\mathrm{Na_2SO_4}$) extracts were concentrated and distilled to afford **16** (4.21 g, 64%). Bp 82-85 °C (11 mm Hg); IR ($\mathrm{CHCl_3}$ cast) 3441 (br m), 2977 (m), 1964 (s), 1175 (s), 1160 (s) $\mathrm{cm^{-1}}$; ^{1}H NMR (400 MHz, $\mathrm{CDCl_3}$) δ 4.10 (dq, 2 H, J = 3.0, 7.2 Hz, $\mathrm{OC\underline{H}_2CH_3}$), 4.12 (m, 1 H, $\mathrm{C\underline{H}(OH)}$), 3.58 (br s, 1 H, $\mathrm{O\underline{H}}$), 2.39 (m, 2 H, $\mathrm{C\underline{H}_2{}^{13}CO}$), 1.20 (t, 3 H, J = 7.2 Hz, $\mathrm{OCH_2C\underline{H}_3}$), 1.16 (d, 3 H, J = 6.2 Hz, $\mathrm{C\underline{H}_3CH(OH)}$); ^{13}C NMR (100 MHz, $\mathrm{CDCl_3}$) δ 172.61 (enriched, $\mathrm{^{13}\underline{C}O}$), 64.13 ($\mathrm{\underline{C}H(OH)}$), 60.44 ($\mathrm{O\underline{C}H_2CH_3}$), 42.83 (d, J = 57.3 Hz, $\mathrm{\underline{C}H_2{}^{13}CO}$), 22.32 ($\mathrm{\underline{C}H_3CH(OH)}$), 13.96 ($\mathrm{OCH_2\underline{C}H_3}$); HRMS (EI) *m/z* (relative intensity) 118.0586 (57), 89.0359 (63).

For unlabeled compound (**16a**): $[\alpha]_D$ +36.0° (*c* 2.18, $\mathrm{CHCl_3}$); IR ($\mathrm{CHCl_3}$ cast) 3451 (br m), 2977 (m), 1735 (s), 1182 (s) $\mathrm{cm^{-1}}$; ^{1}H NMR (200 MHz, $\mathrm{CDCl_3}$) δ 4.05 (q, 2 H, J = 6.9 Hz, $\mathrm{OC\underline{H}_2CH_3}$), 4.01 (m, 1 H, $\mathrm{C\underline{H}(OH)}$), 3.29 (br s, 1 H, $\mathrm{O\underline{H}}$), 2.33 (m, 2 H, $\mathrm{C\underline{H}_2CO}$), 1.15 (t, 3 H, J = 6.9 Hz, $\mathrm{OCH_2C\underline{H}_3}$), 1.11 (d, 3 H, J = 6.1 Hz, $\mathrm{C\underline{H}_3CH(OH)}$); ^{13}C NMR (50 MHz, $\mathrm{CDCl_3}$) δ 172.43 ($\mathrm{\underline{C}O}$), 64.01 ($\mathrm{\underline{C}H(OH)}$), 60.32 ($\mathrm{O\underline{C}H_2CH_3}$), 42.85 ($\mathrm{\underline{C}H_2CO}$), 22.26 ($\mathrm{\underline{C}H_3CH(OH)}$), 13.84 ($\mathrm{OCH_2\underline{C}H_3}$); MS (CI, $\mathrm{NH_3}$) *m/z* (relative intensity)

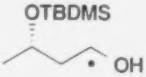
150 (MNH_4^+, 96), 133 (MH^+, 100); MS (EI) *m/z* (relative intensity) 117 (47), 88 (53); Anal. Calcd for $C_6H_{12}O_3$: C, 54.53; H, 9.15. Found: C, 54.33; H, 9.45.



Ethyl (*S*)-[1-^{13}C]-3-(*tert*-butyldimethylsilyloxy)butanoate (**17**) Dr. Zhe Li's procedure[27] was followed. Alcohol **16** (4.12 g, 31.0 mmol), *tert*-butyldimethylsilyl chloride (7.00 g, 46.4 mmol) and imidazole (3.16 g, 46.4 mmol) were dissolved in DMF (100 mL), and the solution was stirred at room temperature overnight. The mixture was diluted with *n*-hexane (200 mL) and then washed with water (4 x 100 mL). The dried (Na_2SO_4) organic layer was concentrated and distilled to give **17** (8.25 g, quantitative). Bp 95-100 °C (8 mm Hg); IR ($CHCl_3$ cast) 2958 (s), 2931 (s), 2858 (m), 1697 (s), 1082 (s), 837 (s) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.27 (m, 1 H, $C\underline{H}(OSi)$), 4.10 (m, 2 H, $OC\underline{H}_2CH_3$), 2.40 (m, 2 H, $C\underline{H}_2{}^{13}CO$), 1.25 (t, 3 H, *J* = 7.1 Hz, $OCH_2C\underline{H}_3$), 1.19 (d, 3 H, *J* = 6.1 Hz, $C\underline{H}_3CH(OSi)$), 0.85 (s, 9 H, $(C\underline{H}_3)_3C$), 0.05 (s, 3 H, $C\underline{H}_3SiCH_3$) , 0.03 (s, 3 H, $CH_3SiC\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.58 (enriched, $^{13}\underline{C}O$), 65.64 ($\underline{C}H(OSi)$), 60.16 ($O\underline{C}H_2CH_3$), 44.95 (d, *J* = 57.3 Hz, $\underline{C}H_2{}^{13}CO$), 25.71 ($(\underline{C}H_3)_3C$), 23.89 ($\underline{C}H_3CH(OSi)$), 17.92 ($(CH_3)_3\underline{C}$), 14.16 ($OCH_2\underline{C}H_3$), -4.54 and -5.06 ($Si(\underline{C}H_3)_2$); MS (CI, NH_3) *m/z* (relative intensity) 265 (MNH_4^+, 2), 248 (MH^+, 100); HRMS (EI) *m/z* (relative intensity) 232.1438 (2), 190.0974 (100).

For unlabeled compound (**17a**): $[\alpha]_D$ +20.1° (*c* 2.65, $CHCl_3$); IR ($CHCl_3$ cast) 2957 (s), 2930 (s), 2857 (m), 1739 (s), 1083 (s), 836 (s) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.27 (m, 1 H, $C\underline{H}(OSi)$), 4.11 (m, 2 H, $OC\underline{H}_2CH_3$), 2.40 (m, 2 H, $C\underline{H}_2CO$), 1.25 (t, 3 H, *J* = 7.2 Hz, $OCH_2C\underline{H}_3$), 1.19 (d, 3 H, *J* = 6.0 Hz, $C\underline{H}_3CH(OSi)$), 0.85 (s, 9 H, $(C\underline{H}_3)_3C$), 0.05 (s, 3 H, $C\underline{H}_3SiCH_3$) , 0.03 (s, 3 H, $CH_3SiC\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.61 ($\underline{C}O$), 65.84 ($\underline{C}H(OSi)$), 60.19 ($O\underline{C}H_2CH_3$), 44.95 ($\underline{C}H_2CO$), 25.70 ($(\underline{C}H_3)_3C$), 23.89 ($\underline{C}H_3CH(OSi)$), 17.93 ($(CH_3)_3\underline{C}$), 14.16 ($OCH_2\underline{C}H_3$), -4.54 and -5.07

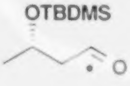
$(Si(\underline{C}H_3)_2)$; MS (CI, NH_3) *m/z* (relative intensity) 247 (MH^+, 100); MS (EI) *m/z* (relative intensity) 231.1415 (4), 189.0946 (100); Anal. Calcd for $C_{12}H_{26}O_3Si$: C, 58.49; H, 10.63. Found: C, 58.46; H, 10.54.



(*S*)-[1-^{13}C]-3-(*tert*-Butyldimethylsilyloxy)butanol (**18**) Dr. Zhe Li's procedure[27] was followed. To a cold (-78 °C) solution of ester **17** (5.42 g, 21.9 mmol) in CH_2Cl_2 (100 mL) was added DIBAL (20.0 mL, 109 mmol) in CH_2Cl_2 (30 mL). The solution was stirred at -78 °C for 1.5 h, warmed to -30 °C and then quenched by adding methanol. The mixture was diluted with ether (200 mL), and washed with saturated aqueous potassium sodium tartrate (4 x 100 mL), brine (2 x 100 mL). The dried (Na_2SO_4) ether layer was concentrated *in vacuo* to give **18** (3.26 g, 73%). IR ($CHCl_3$ cast) 3354 (br m), 2957 (s), 2930 (s), 2858 (m), 1256 (m), 1020 (m), 837 (s) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.10 (m, 1 H, $C\underline{H}(OSi)$), 3.83 (dm, 1 H, $J = 141.9$ Hz, $^{13}C\underline{H}HOH$), 3.72 (dm, 1 H, $J = 141.9$ Hz, $^{13}CH\underline{H}OH$), 2.30 (br s, 1 H, $O\underline{H}$), 1.85-1.55 (m, 2 H, $C\underline{H}_2{}^{13}CH_2OH$), 1.20 (d, 3 H, $J = 6.2$ Hz, $C\underline{H}_3CH(OSi)$), 0.89 (s, 9 H, $(C\underline{H}_3)_3C$), 0.08 (s, 6 H, $Si(C\underline{H}_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.34 ($\underline{C}H(OSi)$), 60.44 (enriched, $\underline{C}H_2OH$), 40.44 (d, $J = 37.2$ Hz, $\underline{C}H_2{}^{13}CH_2OH$), 25.77 ($(\underline{C}H_3)_3C$), 23.39 ($\underline{C}H_3CH(OSi)$), 17.92 ($(CH_3)_3\underline{C}$), -4.38 and -4.99 ($Si(\underline{C}H_3)_2$); MS (CI, NH_3) *m/z* (relative intensity) 206 (MH^+, 100); HRMS (EI) calcd for $^{13}CC_9H_{24}O_2Si$ 205.1579, found 205.1573.

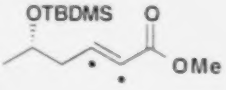
For unlabeled compound (**18a**): $[\alpha]_D$ +23.9° (*c* 2.05, $CHCl_3$); IR ($CHCl_3$ cast) 3350 (br m), 2950 (s), 2925 (s), 2855 (m), 1258 (m), 1060 (m), 855 (s), 775 (s) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.09 (m, 1 H, $C\underline{H}(OSi)$), 3.75 (m, 2 H, $C\underline{H}_2OH$), 2.22 (br s, 1 H, $O\underline{H}$), 1.68 (m, 2 H, $C\underline{H}_2CH_2OH$), 1.18 (d, 3 H, $J = 6.5$ Hz, $C\underline{H}_3CH(OSi)$), 0.88 (s, 9 H, $(C\underline{H}_3)_3C$), 0.08 (s, 6 H, $Si(C\underline{H}_3)_2$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 68.13 ($\underline{C}H(OSi)$), 60.37 ($\underline{C}H_2OH$), 40.80 ($\underline{C}H_2CH_2OH$), 25.83 ($(\underline{C}H_3)_3C$), 23.50 ($\underline{C}H_3CH(OSi)$), 17.96 ($(CH_3)_3\underline{C}$),

-4.35 and -4.91 ($Si(\underline{C}H_3)_2$); MS (CI, NH_3) *m/z* (relative intensity) 205 (MH^+, 100); HRMS (EI) *m/z* (relative intensity) 189.1314 (2), 147.0841 (25), 119.0528 (54); Anal. Calcd for $C_{10}H_{24}O_2Si$: C, 58.77; H, 11.84. Found: C, 58.65; H, 11.98.



(*S*)-[1-^{13}C]-3-(*tert*-Butyldimethylsilyloxy)butanal (19) Dr. Zhe Li's procedure[27] was followed. To a cold (-78 °C) solution of oxalyl chloride (2.36 mL, 26.7 mmol) in CH_2Cl_2 (50 mL) was added DMSO (3.80 mL, 53.4 mmol) in CH_2Cl_2 (15 mL). After 5 min alcohol **18** (3.22 g, 15.7 mmol) in CH_2Cl_2 (20 mL) was added. The mixture was stirred at -78 °C for 45 min before triethylamine (22 mL, 160 mmol) was added, and then warmed to room temperature. The mixture was diluted with ether (200 mL), washed with 1N HCl (3 x 150 mL), brine (150 mL). The dried (Na_2SO_4) ether layer was concentrated and purified by SiO_2 chromatography (5:1 hexane-Et_2O) to afford **19** (1.65 g, 71%). IR ($CHCl_3$ cast) 2958 (s), 2931 (s), 2858 (m), 1673 (s), 1257 (m), 836 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.79 (dt, 1 H, *J* = 172.5, 2.4 Hz, $^{13}C\underline{H}O$), 4.35 (m, 1 H, $C\underline{H}(OSi)$), 2.60-2.41 (m, 2 H, $C\underline{H}_2{}^{13}CHO$), 1.23 (d, 3 H, *J* = 6.2 Hz, $C\underline{H}_3CH(OSi)$), 0.86 (s, 9 H, $(C\underline{H}_3)_3C$), 0.07 (s, 3 H, $C\underline{H}_3SiCH_3$), 0.05 (s, 3 H, $CH_3SiC\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.17 (enriched, $^{13}\underline{C}O$), 64.52 ($\underline{C}H(OSi)$), 52.93 (d, *J* = 39.2 Hz, $\underline{C}H_2{}^{13}CHO$), 25.69 ($(\underline{C}H_3)_3C$), 24.13 ($\underline{C}H_3CH(OSi)$), 17.90 ($(CH_3)_3\underline{C}$), -4.43 and -4.99 ($Si(\underline{C}H_3)_2$); HRMS (EI) *m/z* (relative intensity) 162.0661 (44), 119.0528 (67), 75.0263 (100).

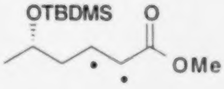
For unlabeled compound (**19a**): $[\alpha]_D$ +14.2° (*c* 1.15, $CHCl_3$); IR ($CHCl_3$ cast) 2956 (s), 2930 (s), 2858 (s), 1715 (s), 1256 (m), 836 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.71 (t, 1 H, *J* = 2.2 Hz, $C\underline{H}O$), 4.28 (m, 1 H, $C\underline{H}(OSi)$), 2.42 (m, 2 H, $C\underline{H}_2CHO$), 1.15 (d, 3 H, *J* = 6.2 Hz, $C\underline{H}_3CH(OSi)$), 0.79 (s, 9 H, $(C\underline{H}_3)_3C$), 0.00 (s, 3 H, $C\underline{H}_3SiCH_3$), -0.02 (s, 3 H, $CH_3SiC\underline{H}_3$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 201.82 ($\underline{C}O$), 64.39 ($\underline{C}H(OSi)$), 52.85 ($\underline{C}H_2CHO$), 25.60 ($(\underline{C}H_3)_3C$), 24.02 ($\underline{C}H_3CH(OSi)$), 17.80 ($(CH_3)_3\underline{C}$), 4.53 and -5.10 ($Si(\underline{C}H_3)_2$); MS (CI, NH_3) *m/z* (relative intensity) 220 (MNH_4^+, 36), 203 (MH^+, 100); HRMS (EI) *m/z* (relative intensity) 161.0630 (47), 119.0526 (67), 75.0267 (100); Anal. Calcd for $C_{10}H_{22}O_2Si$: C, 59.35; H, 10.96. Found: C, 59.19; H, 11.05.



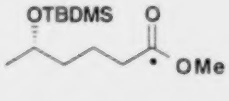
Methyl (*S*)-[2,3-$^{13}C_2$]-5-(*tert*-Butyldimethylsilyloxy)hex-2-enoate (20). Dr. Zhe Li's procedure[27] was followed. Aldehyde **19** (1.62 g, 7.98 mmol) and [2-^{13}C](carbomethoxymethylene)triphenylphosphorane (2.95 g, 8.78 mmol) were dissolved in benzene (60 mL). The mixture was heated under reflux overnight (ca. 16h). The precipitate formed was removed by filtration, and washed with hexane. The filtrate was then concentrated and purified by SiO_2 chromatography (5:1 hexane-Et_2O) to afford **20** (2.00 g, 96%), exclusively as the *E*-isomer. IR (CH_2Cl_2 cast) 2955 (s), 2930 (s), 2858 (m), 1727 (s), 1663 (m), 837 (s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.96 (ddtd, 1 H, *J* = 155.6, 15.7, 7.5, 1.8 Hz, $^{13}C\underline{H}{=}^{13}CHCO$), 5.84 (dd, 1 H, *J* = 162.2, 15.7 Hz, $^{13}CH{=}^{13}C\underline{H}CO$), 3.93 (m, 1 H, $C\underline{H}(OSi)$), 3.73 (s, 3 H, $OC\underline{H}_3$), 2.38-2.26 (m, 2 H, $CH(OSi)C\underline{H}_2$), 1.16 (d, 3 H, *J* = 6.1 Hz, $C\underline{H}_3CH(OSi)$), 0.88 (s, 9 H, $(C\underline{H}_3)_3C$), 0.05 (s, 3 H, $(C\underline{H}_3SiCH_3)$, 0.04 (s, 3 H, $(CH_3SiC\underline{H}_3)$; ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.78 (d, *J* = 56.6 Hz, $\underline{C}O$), 146.27 (d, *J* = 70.5 Hz, enriched, $^{13}\underline{C}H{=}^{13}CHCO$), 122.76 (d, *J* = 70.5 Hz, enriched, $^{13}CH{=}^{13}\underline{C}HCO$), 67.58 ($\underline{C}H(OSi)$), 51.35 ($O\underline{C}H_3$), 42.43 (d, *J* = 43.0 Hz, $CH(OSi)\underline{C}H_2$), 25.78 ($(\underline{C}H_3)_3C$), 23.71 ($\underline{C}H_3CH(OSi)$), 18.05 ($(CH_3)_3\underline{C}$), -4.57 and -4.85 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) *m/z* (relative intensity) 278 (MNH_4^+, 100), 259 (MH^+, 48); HRMS (EI) *m/z* (relative intensity) 245.1478 (5), 203.1012 (90), 159.1202 (85), 133.0686 (100).

For unlabeled compound (**20a**): $[\alpha]_D$ +6.54° (*c* 1.91, $CHCl_3$); IR (neat) 2955 (s), 2931 (s), 2858 (s), 1729 (s), 1660 (m), 837 (s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.96 (dt, 1 H, *J* = 15.8, 7.6 Hz, $C\underline{H}{=}CHCO$), 5.83 (dt, 1 H, *J* = 15.8, 1.5 Hz, $CH{=}C\underline{H}CO$), 3.92

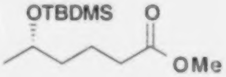
(m, 1 H, $C\underline{H}(OSi)$), 3.72 (s, 3 H, $OC\underline{H}_3$), 2.36-2.26 (m, 2 H, $CH(OSi)C\underline{H}_2$), 1.16 (d, 3 H, J = 6.0 Hz, $C\underline{H}_3CH(OSi)$), 0.88 (s, 9 H, $(C\underline{H}_3)_3C$), 0.04 (s, 3 H, $(C\underline{H}_3SiCH_3)$), 0.03 (s, 3 H, $(CH_3SiC\underline{H}_3)$; ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.81 ($\underline{C}O$), 146.26 ($\underline{C}H{=}CHCO$), 122.78 ($CH{=}\underline{C}HCO$), 67.57 ($\underline{C}H(OSi)$), 51.31 ($O\underline{C}H_3$), 42.42 ($CH(OSi)\underline{C}H_2$), 25.76 ($(\underline{C}H_3)_3C$), 23.69 ($\underline{C}H_3CH(OSi)$), 18.04 ($(CH_3)_3\underline{C}$), -4.59 and -4.87 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) *m/z* (relative intensity) 276 (MNH_4^+, 100), 259 (MH^+, 48); HRMS (EI) *m/z* (relative intensity) 243.1415 (2), 201.0947 (49), 159.1206 (88); Anal. Calcd for $C_{13}H_{26}O_3Si$: C, 60.42; H, 10.14. Found: C, 60.48; H, 10.28.



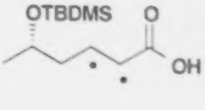
Methyl (*S*)-[2,3-$^{13}C_2$]-5-(*tert*-Butyldimethylsilyloxy)hexanoate (**21**) The same procedure as for unlabeled compound **21b** was used. IR ($CHCl_3$ cast) 2955 (s), 2930 (s), 2858 (s), 1744 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.80 (m, 1 H, $C\underline{H}(OSi)$), 3.67 (s, 3 H, $OC\underline{H}_3$), 2.30 (dm, 2 H, J = 127.8 Hz, $^{13}C\underline{H}_2CO$), 1.81 (m, 1 H, $^{13}C\underline{H}H^{13}CH_2CO$), 1.60-1.35 (m, 3 H, $^{13}CH\underline{H}^{13}CH_2CO + CH(OSi)C\underline{H}_2$), 1.12 (d, 3 H, J = 6.1 Hz, $C\underline{H}_3CH(OSi)$), 0.88 (s, 9 H, $(C\underline{H}_3)_3C$), 0.05 (s, 6 H, $(CH_3)_2Si$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.21 (d, J = 57.3 Hz, $\underline{C}O$), 68.14 ($\underline{C}H(OSi)$), 51.40 ($O\underline{C}H_3$), 38.97 (d, J = 35.0 Hz, $CH(OSi)\underline{C}H_2$), 34.09 (d, J = 34.1 Hz, enriched, $^{13}CH_2{}^{13}\underline{C}H_2CO$), 25.87 ($(\underline{C}H_3)_3C$), 23.69 ($\underline{C}H_3CH(OSi)$), 21.19 (d, J = 34.1 Hz, enriched, $^{13}\underline{C}H_2{}^{13}CH_2CO$), 18.09 ($(CH_3)_3\underline{C}$), -4.42 and -4.77 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) *m/z* (relative intensity) 280 (MNH_4^+, 1), 263 (MH^+, 61); HRMS (EI) *m/z* (relative intensity) 231.1695 (11), 205.1168 (29).



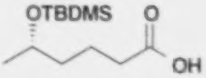
Methyl (*S*)-[1-^{13}C]-5-(*tert*-Butyldimethylsilyloxy)hexanoate (**21a**). The same procedure as for unlabeled compound **21b** was used. IR ($CHCl_3$ cast) 2955 (m), 2930 (m), 2858 (m), 1701 (s), 1155 (s), 836 (s) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 3.77 (m, 1 H, $\mathrm{C\underline{H}(OSi)}$), 3.63 (d, 3 H, J = 3.9 Hz, $\mathrm{OC\underline{H}_3}$), 2.28 (q, 2 H, J = 7.3 Hz, $\mathrm{CH(OSi)C\underline{H}_2}$), 1.65 (m, 2 H, $\mathrm{C\underline{H}_2{}^{13}CO}$), 1.40 (m, 2 H, $\mathrm{C\underline{H}_2CH_2{}^{13}CO}$), 1.10 (d, 3 H, J = 6.0 Hz, $\mathrm{C\underline{H}_3CH(OSi)}$), 0.86 (s, 9 H, $\mathrm{(C\underline{H}_3)_3C}$), 0.02 (s, 6 H, $\mathrm{(Si(C\underline{H}_3)_2}$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 173.96 (enriched, $\mathrm{{}^{13}\underline{C}O}$), 68.09 ($\mathrm{\underline{C}H(OSi)}$), 51.27 ($\mathrm{O\underline{C}H_3}$), 38.92 ($\mathrm{CH(OSi)\underline{C}H_2}$), 33.98 (d, J = 57.4 Hz, $\mathrm{\underline{C}H_2{}^{13}CO}$), 25.76 ($\mathrm{(\underline{C}H_3)_3C}$), 23.62 ($\mathrm{\underline{C}H_3CH(OSi)}$), 21.12 ($\mathrm{\underline{C}H_2CH_2{}^{13}CO}$), 18.02 ($\mathrm{(CH_3)_3\underline{C}}$), -4.58 and -4.85 ($\mathrm{(\underline{C}H_3)_2Si}$); MS (CI, NH$_3$) *m/z* (relative intensity) 279 ($\mathrm{MNH_4^+}$, 11), 262 (MH$^+$, 94); HRMS (EI) *m/z* (relative intensity) 246.1605 (4), 230.1658 (12), 204.1137 (66), 172.0877 (100).



Methyl (*S*)-5-(*tert*-Butyldimethylsilyloxy)hexanoate (21b) Under an atmosphere of H$_2$, the unsaturated ester **20a** (1.02 g, 3.95 mmol) in EtOAc (25 mL) was stirred in the presence of palladium catalyst (10% Pd/C, 100 mg) for 2 h. The mixture was then filtered, and washed with EtOAc. After the concentration *in vacuo*, the crude product was purified with SiO$_2$ column chromatography (5% Et$_2$O in hexane) to give **21b** as an oil (0.94 g, 92%). $[\alpha]_D$ +10.0° (c 1.18, CHCl$_3$); IR (CHCl$_3$ cast) 2965 (s), 2945 (s), 2856 (s), 1744 (s), 1254 (s), 836 (s) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 3.80 (m, 1 H, $\mathrm{C\underline{H}(OSi)}$), 3.66 (s, 3 H, $\mathrm{OC\underline{H}_3}$), 2.31 (t, 2 H, J = 7.4 Hz, $\mathrm{C\underline{H}_2CO}$), 1.79-1.52 (m, 2 H, $\mathrm{CH(OSi)C\underline{H}_2}$), 1.48-1.36 (m, 2 H, $\mathrm{C\underline{H}_2CH_2CO}$), 1.12 (d, 3 H, J = 6.0 Hz, $\mathrm{C\underline{H}_3CH(OSi)}$), 0.88 (s, 9 H, $\mathrm{(C\underline{H}_3)_3C}$), 0.04 (s, 6 H, $\mathrm{(C\underline{H}_3)_2Si}$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 174.09 ($\mathrm{\underline{C}O}$), 68.16 ($\mathrm{\underline{C}H(OSi)}$), 51.39 ($\mathrm{O\underline{C}H_3}$), 38.98 ($\mathrm{CH(OSi)\underline{C}H_2}$), 34.08 ($\mathrm{\underline{C}H_2CO}$), 25.86 ($\mathrm{(\underline{C}H_3)_3C}$), 23.68 ($\mathrm{\underline{C}H_3CH(OSi)}$), 21.20 ($\mathrm{\underline{C}H_2CH_2CO}$), 18.09 ($\mathrm{(CH_3)_3\underline{C}}$), -4.42 and -4.77 ($\mathrm{(\underline{C}H_3)_2Si}$); MS (CI, NH$_3$) *m/z* (relative intensity) 278 ($\mathrm{MNH_4^+}$, 3), 261 (MH$^+$, 65); HRMS (EI) *m/z* (relative intensity) 245.1567 (4), 229.1618 (14), 203.1101 (91); Anal. Calcd for $\mathrm{C_{13}H_{28}O_3Si}$: C, 59.95; H, 10.84. Found: C, 59.94; H, 11.01.

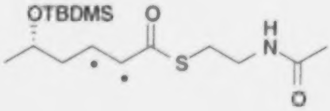


Methyl (*S*)-[2,3-$^{13}C_2$]-5-(*tert*-Butyldimethylsilyloxy)hexanoic Acid (22) The same procedure as for unlabeled compound **22a** was used. IR ($CHCl_3$ cast) 3025 (br m), 2957 (s), 2930 (s), 2858 (m), 1712 (s), 1256 (s), 836 (m), 775 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 10.97 (br s, 1 H, $COO\underline{H}$), 3.81 (m, 1 H, $C\underline{H}(OSi)$), 2.35 (dm, 2 H, J = 127.5 Hz, $^{13}C\underline{H}_2CO$), 1.68 (dm, 2 H, J = 130.6 Hz, $^{13}C\underline{H}_2{}^{13}CH_2CO$), 1.47 (m, 2 H, $CH(OSi)C\underline{H}_2$), 1.14 (d, 3 H, J = 6.1 Hz, $C\underline{H}_3CH(OSi)$), 0.89 (s, 9 H, $(C\underline{H}_3)_3C$), 0.06 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.91 (d, J = 57.4 Hz, $\underline{C}O$), 68.17 ($\underline{C}H(OSi)$), 38.82 (d, J = 35.0 Hz, $CH(OSi)\underline{C}H_2$), 34.06 (d, J = 34.5 Hz, enriched, $^{13}CH_2{}^{13}\underline{C}H_2CO$), 25.86 ($(\underline{C}H_3)_3C$), 23.66 ($\underline{C}H_3CH(OSi)$), 20.90 (d, J = 34.5 Hz, enriched, $^{13}\underline{C}H_2{}^{13}CH_2CO$), 18.09 ($(CH_3)_3\underline{C}$), -4.42 and -4.78 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) *m/z* (relative intensity) 266 (MNH_4^+, 3), 249 (MH^+, 100); HRMS (EI) *m/z* (relative intensity) 233.1479 (1), 215.1377 (4), 191.1014 (25), 173.0907 (100).

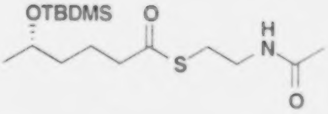


Methyl (*S*)-5-(*tert*-Butyldimethylsilyloxy)hexanoic Acid (22a) To a solution of **21b** (210 mg, 0.854 mmol) in THF (8 mL) and H_2O (2 mL) was added 1.0 M NaOH (1.7 mL) and tetramethylammonium hydroxide (20% solution in CH_3OH, 0.1 mL). The mixture was stirred at 30 °C for 30 min, then half of the solvent was evaporated *in vacuo*. The residue was then further stirred at 30 °C overnight. The reaction solution was then acidifed to pH 2, extracted with $CHCl_3$ (5 x 20 mL), and the combined extracts were dried (Na_2SO_4). Purification by SiO_2 column chromatography (25% EtOAc in hexane) gave **22a** (195 mg, 98%) as an oil. $[\alpha]_D$ +11.9° (*c* 1.39, $CHCl_3$); IR (neat film) 3039 (br m), 2958 (s), 2931 (s), 2858 (s), 1712 (s), 1256 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ

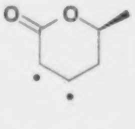
3.82 (m, 1 H, CH(OSi)), 2.36 (t, 2 H, J = 7.2 Hz, $C\underline{H}_2CO$), 1.68 (m, 2 H, $C\underline{H}_2CH_2CO$), 1.47 (m, 2 H, $CH(OSi)C\underline{H}_2$), 1.13 (d, 3 H, J = 6.2 Hz, $C\underline{H}_3CH(OSi)$), 0.89 (s, 9 H, $(C\underline{H}_3)_3C$), 0.06 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.97 ($\underline{C}O$), 68.16 ($\underline{C}H(OSi)$), 38.84 ($CH(OSi)\underline{C}H_2$), 34.00 ($\underline{C}H_2CO$), 25.87 ($(\underline{C}H_3)_3C$), 23.68 ($\underline{C}H_3CH(OSi)$), 20.92 ($\underline{C}H_2CH_2CO$), 18.09 ($(CH_3)_3\underline{C}$), -4.41 and -4.71 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) *m/z* (relative intensity) 247(MH^+, 100); MS (EI) *m/z* (relative intensity) 189.0949 (15); Anal. Calcd for $C_{12}H_{26}O_3Si$: C, 58.49; H, 10.63. Found: C, 58.76; H, 10.86.



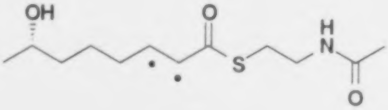
NAC (*S*)-[2,3-$^{13}C_2$]-5-(*tert*-Butyldimethylsilyloxy)hexanoate (23) The same procedure as for unlabeled compound **23a** was used. IR ($CHCl_3$ cast) 3285 (m), 2929 (s), 2857 (m), 1692 (s), 1655 (s), 836 (m), 755 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.98 (br s, 1 H, $N\underline{H}$), 3.78 (m, 1 H, $C\underline{H}(OSi)$), 3.42 (q, 2 H, J = 6.2 Hz, $C\underline{H}_2NH$), 3.01 (t, 2 H, J = 6.4 Hz, $SC\underline{H}_2$), 2.56 (dm, 2 H, J = 128.7 Hz, $^{13}C\underline{H}_2CO$), 1.96 (s, 3 H, $COC\underline{H}_3$), 1.68 (dm, 2 H, J = 132.0 Hz, $^{13}C\underline{H}_2{}^{13}CH_2CO$), 1.48-1.35 (m, 2 H, $CH(OSi)C\underline{H}_2$), 1.11 (d, 3 H, J = 6.1 Hz, $C\underline{H}_3CH(OSi)$), 0.87 (s, 9 H, $(C\underline{H}_3)_3C$), 0.04 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.98 (d, J = 46.0 Hz, $\underline{C}OS$), 170.24 ($CH_3\underline{C}O$), 68.00 ($\underline{C}H(OSi)$), 44.05 (d, J = 33.9 Hz, enriched, $^{13}\underline{C}H_2CO$), 39.72 ($\underline{C}H_2NH$), 38.64 (d, J = 35.2 Hz, $CH(OSi)\underline{C}H_2$), 28.38 ($S\underline{C}H_2$), 25.84 ($(\underline{C}H_3)_3C$), 23.70 ($\underline{C}H_3CH(OSi)$), 23.15 ($CO\underline{C}H_3$), 21.85 (d, J = 33.9 Hz, enriched, $^{13}\underline{C}H_2{}^{13}CH_2CO$), 18.05 ($(CH_3)_3\underline{C}$), -4.40 and -4.77 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) *m/z* (relative intensity) 367 (MNH_4^+, 13), 350 (MH^+, 98); HRMS (EI) *m/z* (relative intensity) 334.1786 (3), 292.1311 (58).



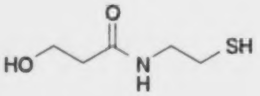
NAC (*S*)-5-(*tert*-Butyldimethylsilyloxy)hexanoate (23a) The procedure for the preparation of **7a** was adopted. Thus, acid **22a** (150 mg, 0.61 mmol) was condensed with *N*-acetylcysteamine (80 mg, 0.67 mmol) in the presence of DCC (138 mg, 6.7 mmol) and 4-dimethylaminopyridine (5 mg) to afford **23a** (179 mg, 85%). $[\alpha]_D$ +8.98° (*c* 1.18, $\mathrm{CHCl_3}$); IR ($\mathrm{CHCl_3}$ cast) 3287 (m), 2956 (s), 2930 (s), 2857 (s), 1693 (s), 1656 (s), 1553 (m) cm^{-1}; ^{1}H NMR (300 MHz, $\mathrm{CDCl_3}$) δ 6.00 (br s, 1 H, $\mathrm{N\underline{H}}$), 3.78 (m, 1 H, $\mathrm{C\underline{H}(OSi)}$), 3.41 (q, 2 H, *J* = 6.2 Hz, $\mathrm{C\underline{H}_2NH}$), 3.01 (t, 2 H, *J* = 6.4 Hz, $\mathrm{SC\underline{H}_2}$), 2.57 (t, 2 H, *J* = 7.5 Hz, $\mathrm{C\underline{H}_2CO}$), 1.95 (s, 3 H, $\mathrm{COC\underline{H}_3}$), 1.85-1.55 (m, 2 H, $\mathrm{C\underline{H}_2CH_2CO}$)), 1.50-1.30 (m, 2 H, $\mathrm{CH(OSi)C\underline{H}_2}$), 1.11 (d, 3 H, *J* = 6.2 Hz, $\mathrm{C\underline{H}_3CH(OSi)}$), 0.87 (s, 9 H, $\mathrm{(C\underline{H}_3)_3C}$), 0.04 (s, 6 H, $\mathrm{(C\underline{H}_3)_2Si}$); ^{13}C NMR (75 MHz, $\mathrm{CDCl_3}$) δ 199.93 ($\mathrm{\underline{C}OS}$), 170.21 ($\mathrm{\underline{C}OCH_3}$), 67.99 ($\mathrm{\underline{C}H(OSi)}$)), 44.04 ($\mathrm{\underline{C}H_2CO}$), 39.69 ($\mathrm{\underline{C}H_2NH}$), 38.64 ($\mathrm{CH(OSi)\underline{C}H_2}$), 28.38 ($\mathrm{S\underline{C}H_2}$), 25.83 ($\mathrm{(\underline{C}H_3)_3C}$), 23.68 ($\mathrm{\underline{C}H_3CH(OSi)}$)), 23.12 ($\mathrm{CO\underline{C}H_3}$), 21.85 ($\mathrm{\underline{C}H_2CH_2CO}$), 18.03 $\mathrm{(CH_3)_3\underline{C}}$), -4.41 and -4.78 ($\mathrm{(\underline{C}H_3)_2Si}$); MS (CI, $\mathrm{NH_3}$) *m/z* (relative intensity) 365 ($\mathrm{MNH_4^+}$, 19), 348 ($\mathrm{MH^+}$, 75); HRMS (EI) *m/z* (relative intensity) 290.1238 (58); Anal. Calcd for $\mathrm{C_{16}H_{33}NO_3SSi}$: C, 55.29; H, 9.57, N, 4.03. Found: C, 55.56; H, 9.87, N, 4.21.



(*S*)-Tetrahydro-6-methyl-2*H*-pyran-2-one (24) A solution of **8** (45 mg) in $\mathrm{CDCl_3}$ (0.6 mL) was maintained in a NMR tube at room temperature for a week. The following data were recorded for the cyclized product **24**. ^{1}H NMR (300 MHz, $\mathrm{CDCl_3}$) δ 4.42 (m, 1 H, $\mathrm{C\underline{H}(O)}$), 2.82-1.35 (m, 6 H), 1.34 (d, 3 H, *J* = 6.3 Hz, $\mathrm{C\underline{H}_3CH(O)}$); ^{13}C NMR (75 MHz, $\mathrm{CDCl_3}$) δ 171.82 (d, *J* = 51.3 Hz, $\mathrm{\underline{C}O}$), 76.93 (m, $\mathrm{\underline{C}H(O)}$), 29.09 (d, *J* = 31.9 Hz, enriched, $^{13}\underline{C}H_2CO$, the resonance for C-4 was obscured by these enriched signals), 21.58 ($\underline{C}H_3$), 18.35 (d, J = 31.9 Hz, enriched, $^{13}\underline{C}H_2{}^{13}CH_2CO$).

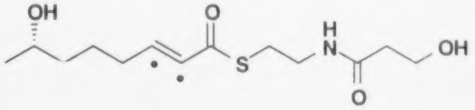


NAC (*S*)-[2,3-$^{13}C_2$]-7-hydroxyoctanoate (27) The method for the conversion of **23a** to **8a** was adopted. Thus, **35** (392 mg, 1.04 mmol) was deprotected with boron trifluoride diethyl etherate (1.5 mL, 12 mmol) in CH_2Cl_2 (15 mL) to give alcohol **27** (255 mg, 93%). $[\alpha]_D$ +3.90° (c 1.43, $CHCl_3$); IR ($CHCl_3$ cast) 3286 (br m), 2930 (s), 2857 (m), 1693 (s), 1646 (s), 1556 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.00 (br s, 1 H, $N\underline{H}$), 3.79 (m, 1 H, $C\underline{H}(OH)$), 3.42 (q, 2 H, J = 6.3 Hz, $NHC\underline{H}_2$), 3.02 (t, 2 H, J = 6.4 Hz, $C\underline{H}_2S$), 2.57 (dm, 2 H, J = 130.1 Hz, $^{13}C\underline{H}_2CO$), 1.96 (s, 3 H, $COC\underline{H}_3$), 1.75 (br s, 1 H, $O\underline{H}$), 1.66 (dm, 2 H, J = 134.6 Hz, $^{13}C\underline{H}_2{}^{13}CH_2CO$), 1.50-1.30 (m, 6 H, $CH(OH)C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 1.18 (d, 3 H, J = 6.2 Hz, $C\underline{H}_3CH(OH)$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.97 (d, J = 45.3 Hz, $\underline{C}OS$), 170.38 ($\underline{C}OCH_3$), 67.72 ($\underline{C}H(OH)$), 43.88 (d, J = 33.4 Hz, enriched, $^{13}\underline{C}H_2CO$), 39.55 ($NH\underline{C}H_2$), 38.89 ($CH(OH)\underline{C}H_2$), 28.77 (d, J = 34.6 Hz, $\underline{C}H_2{}^{13}CH_2$), 28.38 ($\underline{C}H_2S$), 25.46 (d, J = 33.4 Hz, enriched, $^{13}\underline{C}H_2{}^{13}CH_2CO$), 25.37 ($CH(OH)CH_2\underline{C}H_2$), 23.48 ($\underline{C}H_3CH(OH)$), 23.05 ($CO\underline{C}H_3$); MS (CI, NH_3) m/z (relative intensity) 395 (MNH_4^+, 7), 378 (MH^+, 100); HRMS (EI) m/z (relative intensity) 362.2120 (4), 320.1626 (100).



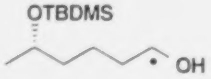
***N*-(3-Hydroxypropionyl)cysteamine (28)** To a stirring solution of disulfide **39** (761 mg, 2 mmol) in dry methanol (15 mL), under argon, was carefully added sodium amalgam (3% Na, 30 g). After stirring at room temperature for 1 h, the mixture was then filtered into a mixture of 1N HCl (20 mL) and CH_2Cl_2 (20 mL), extracted immediately with

CH_2Cl_2 (3 x 50 mL), and dried (Na_2SO_4). After the evaporation of solvent the crude product was purified by SiO_2 column chromatography (9:1 $CHCl_3$-CH_3OH) to afford **28** (167 mg, 28%). IR (CH_2Cl_2 cast) 3302 (br s), 2934 (m), 2550 (w), 1652 (s), 1557 (s), 1052 (m) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.62 (br s, 1 H, $N\underline{H}$), 3.88 (t, 2 H, *J* = 5.5 Hz, $HOC\underline{H}_2$), 3.44 (q, 2 H, *J* = 6.3 Hz, $NHC\underline{H}_2$), 3.27 (br s, 1 H, $O\underline{H}$), 2.67 (dt, 2 H, *J* = 8.5, 6.4 Hz, $C\underline{H}_2SH$), 2.45 (t, 2 H, *J* = 5.5 Hz, $C\underline{H}_2CO$), 1.42 (t, 1 H, *J* = 8.4 Hz, $S\underline{H}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.62 ($CH_2\underline{C}O$), 58.73 ($HO\underline{C}H_2$), 42.22 ($NH\underline{C}H_2$), 38.17 ($\underline{C}H_2CO$), 24.48 ($\underline{C}H_2SH$); HRMS (EI) *m/z* (relative intensity) calcd for $C_5H_{11}NO_2S$ 149.0511, found 149.0508 (M^+, 21).



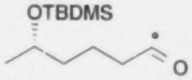
N-(3-Hydroxypropionyl)cysteaminyl (S)-[2,3-$^{13}C_2$]-7-Hydroxyoct-2-enoate (**29**) The method for the conversion of **23a** to **8a** was used. Thus, **42** (67.2 mg, 0.166 mmol) was deprotected with boron trifluoride diehtyl etherate (0.4 mL, 3.2 mmol) in CH_2Cl_2 (15 mL) to give alcohol **29** (31.0 mg, 64%). IR ($CHCl_3$ cast) 3301 (br m), 2925 (s), 2854 (m), 1652 (s), 1548 (m), 1045 (m) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.92 (ddt, 1 H, *J* = 153.8, 15.8, 6.7 Hz, $^{13}C\underline{H}={}^{13}CHCO$), 6.46 (br s, 1 H, $N\underline{H}$), 6.12 (dd, 1 H, *J* = 161.3, 15.8 Hz, $^{13}C\underline{H}CO$), 3.86 (t, 2 H, *J* = 5.5 Hz, $C\underline{H}_2OH$), 3.82 (m, 1 H, $C\underline{H}(OH)$), 3.48 (q, 2 H, *J* = 6.1 Hz, $C\underline{H}_2NH$), 3.10 (t, 2 H, *J* = 6.3 Hz, $SC\underline{H}_2$), 2.63 (br s, 2 H, 2 x $O\underline{H}$), 2.42 (t, 2 H, *J* = 5.4 Hz, $COC\underline{H}_2$), 2.30-2.16 (m, 2 H, $C\underline{H}_2{}^{13}CH$), 1.68-1.40 (m, 4 H, $CH(OH)C\underline{H}_2C\underline{H}_2$), 1.20 (d, 3 H, *J* = 6.2 Hz, $C\underline{H}_3CH(OH)$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.37 (d, *J* = 60.0 Hz, $\underline{C}OS$), 172.69 ($\underline{C}OCH_2$), 146.31 (d, *J* = 69.4 Hz, enriched, $^{13}\underline{C}H={}^{13}CHCO$), 128.48 (d, *J* = 69.4 Hz, enriched, $^{13}\underline{C}HCO$), 67.65 ($\underline{C}H(OH)$), 58.77 ($\underline{C}H_2OH$), 39.53 ($\underline{C}H_2NH$), 38.46 ($CH(OH)\underline{C}H_2$), 38.06 ($CO\underline{C}H_2$), 32.08 (d, *J* = 41.3 Hz, $CH_2{}^{13}CH$), 28.16 ($S\underline{C}H_2$), 24.07 ($\underline{C}H_2CH_2{}^{13}CH$), 23.57 ($\underline{C}H_3CH(OH)$); MS (CI, NH_3) *m/z* (relative intensity) 309

(MNH$_4^+$, 24), 292 (100); HRMS (EI) *m/z* (relative intensity) 248.1287 (3), 234.1080 (4), 149.0506 (80).



(*S*)-[1-^{13}C]-5-(*tert*-Butyldimethylsilyloxy)hexanol (**30**) The procedure employed for the conversion of **17** to **18** was followed. Thus, ester **21a** (1.76 g, 6.75 mmol) was reduced with DIBAL (6.0 mL, 34 mmol) in CH$_2$Cl$_2$ (50 mL) to give alcohol **30** (1.13g, 72%). $[\alpha]_D$ +12.8° (*c* 1.23, CHCl$_3$); IR (CHCl$_3$ cast) 3343 (br m), 2954 (s), 2930 (s), 2858 (s), 1255 (m), 1047 (m), 835 (s) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 3.79 (m, 1 H, $\mathrm{C\underline{H}(OSi)}$), 3.63 (dt, 2 H, *J* = 140.8, 6.6 Hz, $\mathrm{^{13}C\underline{H}_2OH}$), 1.69 (br s, 1 H, $\mathrm{O\underline{H}}$), 1.62-1.51 (m, 2 H, $\mathrm{C\underline{H}_2{}^{13}CH_2OH}$), 1.51-1.30 (m, 4 H, $\mathrm{CH(OSi)C\underline{H}_2C\underline{H}_2}$), 1.12 (d, 3 H, *J* = 6.1 Hz, $\mathrm{C\underline{H}_3CH(OSi)}$), 0.88 (s, 9 H, $\mathrm{(C\underline{H}_3)_3C}$), 0.04 (s, 6 H, $\mathrm{(Si(C\underline{H}_3)_2)}$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 68.51 ($\mathrm{\underline{C}H(OSi)}$), 62.86 (enriched, $\mathrm{^{13}\underline{C}H_2OH}$), 39.37 ($\mathrm{CH(OSi)\underline{C}H_2}$), 32.61 (d, *J* = 37.6 Hz, $\mathrm{\underline{C}H_2{}^{13}CH_2OH}$), 25.88 ($\mathrm{(\underline{C}H_3)_3C}$), 23.73 ($\mathrm{\underline{C}H_3CH(OSi)}$), 21.85 ($\mathrm{\underline{C}H_2CH_2{}^{13}CH_2OH}$), 18.11 ($\mathrm{(CH_3)_3\underline{C}}$), -4.43 and -4.74 ($\mathrm{(\underline{C}H_3)_2Si}$); MS (CI, NH$_3$) *m/z* (relative intensity) 234 (MH$^+$, 100); HRMS (EI) *m/z* (relative intensity) 176.1188 (7), 159.1206 (14), 119.0529 (38). Anal. Calcd for ^{13}CC$_{11}$H$_{28}$O$_2$Si: C, 62.17; H, 12.09. Found: C, 62.10; H, 12.35.

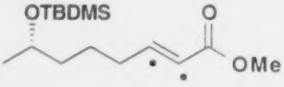
For unlabeled compound (**30a**): IR (CHCl$_3$ cast) 3347 (br m), 2930 (s), 2858 (s), 1255 (m), 1060 (m), 835 (s) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 3.79 (m, 1 H, $\mathrm{C\underline{H}(OSi)}$), 3.65 (t, 2 H, *J* = 6.6 Hz, $\mathrm{C\underline{H}_2OH}$), 1.65-1.30 (m, 7 H, $\mathrm{C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_2O\underline{H}}$), 1.13 (d, 3 H, *J* = 6.0 Hz, $\mathrm{C\underline{H}_3CH(OSi)}$), 0.89 (s, 9 H, $\mathrm{(C\underline{H}_3)_3C}$), 0.05 (s, 6 H, $\mathrm{(Si(C\underline{H}_3)_2)}$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 68.51 ($\mathrm{\underline{C}H(OSi)}$), 62.99 ($\mathrm{\underline{C}H_2OH}$), 39.41 ($\mathrm{CH(OSi)\underline{C}H_2}$), 32.83 ($\mathrm{\underline{C}H_2CH_2OH}$), 25.91 ($\mathrm{(\underline{C}H_3)_3C}$), 23.75 ($\mathrm{\underline{C}H_3CH(OSi)}$), 21.85 ($\mathrm{\underline{C}H_2CH_2CH_2OH}$), 18.14 ($\mathrm{(CH_3)_3\underline{C}}$), -4.39 and -4.70 ($\mathrm{(\underline{C}H_3)_2Si}$); MS (CI, NH$_3$) *m/z* (relative intensity) 233 (MH$^+$, 100); HRMS (EI) *m/z* (relative intensity) 175.1155 (16), 159.1206 (15), 119.0529 (77). Anal. Calcd for $C_{12}H_{28}O_2Si$: C, 62.01; H, 12.14. Found: C, 62.26; H, 12.39.



(*S*)-[1-^{13}C]-5-(*tert*-Butyldimethylsilyloxy)hexanal (**31**). The procedure employed for the conversion of **18** to **19** was used. Thus, alcohol **30** (1.10 g, 4.71 mmol) was oxidized under Swern conditions to afford aldehyde **31** (0.962 g, 88%). $[\alpha]_D$ +15.3° (*c* 1.76, $CHCl_3$); IR (neat) 2956 (s), 2931 (s), 2857 (s), 1688 (s), 1255 (m), 836 (s) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.75 (dt, 1 H, *J* = 170.0, 1.8 Hz, $^{13}C\underline{H}O$), 3.81 (m, 1 H, $C\underline{H}(OSi)$), 2.43 (m, 2 H, $C\underline{H}_2{}^{13}CHO$), 1.80-1.46 (m, 2 H, $CH(OSi)C\underline{H}_2$), 1.44 (m, 2 H, $C\underline{H}_2CH_2{}^{13}CHO$), 1.13 (d, 3 H, *J* = 6.1 Hz, $C\underline{H}_3CH(OSi)$), 0.88 (s, 9 H, $(C\underline{H}_3)_3C$), 0.05 (s, 3 H, $(C\underline{H}_3SiCH_3)$, 0.04 (s, 3 H, $(CH_3SiC\underline{H}_3)$; ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.64 (enriched, $^{13}\underline{C}O$), 68.14 ($\underline{C}H(OSi)$), 43.86 (d, *J* = 38.4 Hz, $\underline{C}H_2{}^{13}CHO$), 38.94 ($CH(OSi)\underline{C}H_2$), 25.85 ($(\underline{C}H_3)_3C$), 23.68 ($\underline{C}H_3CH(OSi)$), 18.32 ($\underline{C}H_2CH_2{}^{13}CHO$), 18.08 ($(CH_3)_3\underline{C}$), -4.40 and -4.77 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) *m/z* (relative intensity) 249 (MNH_4^+, 3), 232 (MH^+, 68); HRMS (EI) *m/z* (relative intensity) 216.1496 (4), 174.1029 (46), 132.0557 (31). Anal. Calcd for $^{13}CC_{11}H_{26}O_2Si$: C, 62.71; H, 11.28. Found: C, 62.47; H, 11.32.

For unlabeled compound (**31a**): IR ($CHCl_3$ cast) 2956 (s), 2930 (s), 2857 (s), 1728 (s), 1255 (m), 836 (s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.77 (t, 1 H, *J* = 1.8 Hz, $C\underline{H}O$), 3.81 (m, 1 H, $C\underline{H}(OSi)$), 2.44 (dt, 2 H, *J* = 1.8, 7.3 Hz, $C\underline{H}_2CHO$), 1.80-1.55 (m, 2 H, $CH(OSi)C\underline{H}_2$), 1.43 (m, 2 H, $C\underline{H}_2CH_2CHO$), 1.14 (d, 3 H, *J* = 6.0 Hz, $C\underline{H}_3CH(OSi)$), 0.89 (s, 9 H, $(C\underline{H}_3)_3C$), 0.06 (s, 3 H, $(C\underline{H}_3SiCH_3)$, 0.05 (s, 3 H, $(CH_3SiC\underline{H}_3)$; ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.64 ($\underline{C}O$), 68.16 ($\underline{C}H(OSi)$), 43.91 ($CH(OSi)\underline{C}H_2$), 38.97 ($\underline{C}H_2CHO$), 25.87 ($(\underline{C}H_3)_3C$), 23.71 ($\underline{C}H_3CH(OSi)$), 18.35 ($\underline{C}H_2CH_2CHO$), 18.10 ($(CH_3)_3\underline{C}$), -4.36 and -4.75 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) *m/z* (relative intensity) 248 (MNH_4^+, 3), 231 (MH^+, 72);

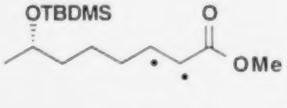
HRMS (EI) *m/z* (relative intensity) 215.1441 (1), 189.0946 (28), 171.0842 (100). Anal. Calcd for $C_{12}H_{26}O_2Si$: C, 62.55; H, 11.37. Found: C, 62.19; H, 11.54.



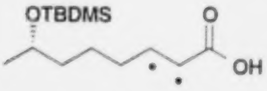
Methyl (*E* and *Z*)-(*S*)-[2,3-$^{13}C_2$]-7-(*tert*-Butyldimethylsilyloxy)oct-2-enoate (**32**) The procedure employed for the conversion of **19** to **20** was followed. Thus, aldehyde **31** (881 mg, 3.81 mmol) was coupled with [2-^{13}C](carbomethoxymethylene)-triphenylphosphorane (1.66 g, 4.96 mmol) to give **32** as a mixture of *E*-isomer (579 mg, 53%) and *Z*-isomer (329 mg, 30%) after SiO_2 chromatography. For *E*-isomer $[\alpha]_D$ +11.0° (*c* 2.08, $CHCl_3$); IR (neat) 2952 (s), 2930(s), 2857 (m), 1727 (s), 1256 (m), 836 (s) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.97 (ddt, 1 H, *J* = 154.1, 15.5, 7.1 Hz, $^{13}C\underline{H}{=}^{13}CHCO$), 5.82 (ddt, 1 H, *J* = 160.1, 15.5, 1.5 Hz, $^{13}CH{=}^{13}C\underline{H}CO$), 3.80 (m, 1 H, $C\underline{H}(OSi)$), 3.71 (s, 3 H, $OC\underline{H}_3$), 2.21 (m, 2 H, $C\underline{H}_2{}^{13}CH$), 1.75-1.32 (m, 4 H, $CH(OSi)C\underline{H}_2C\underline{H}_2$), 1.12 (d, 3 H, *J* = 6.1 Hz, $C\underline{H}_3CH(OSi)$), 0.89 (s, 9 H, $(C\underline{H}_3)_3C$), 0.05 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 166.95 (d, *J* = 75.5 Hz, $\underline{C}O$), 149.19 (d, *J* = 70.5 Hz, enriched, $^{13}\underline{C}H{=}^{13}CHCO$), 120.59 (d, *J* = 70.5 Hz, enriched, $^{13}CH{=}^{13}\underline{C}HCO$), 67.98 ($\underline{C}H(OSi)$), 51.03 ($O\underline{C}H_3$), 38.86 ($CH(OSi)\underline{C}H_2$), 32.02 (d, *J* = 41.3 Hz, $\underline{C}H_2{}^{13}CH$), 25.68 ($(\underline{C}H_3)_3C$), 24.00 ($CH(OSi)CH_2\underline{C}H_2$), 23.66 ($\underline{C}H_3CH(OSi)$), 17.88 ($(CH_3)_3\underline{C}$), -4.53 and -4.89 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) 306 (MNH_4^+, 85), 289 (MH^+, 53); MS (EI) 273.1798 (3), 231.1322 (100).

For *Z*-isomer $[\alpha]_D$ +8.64° (*c* 1.77, $CHCl_3$); IR (CH_2Cl_2 cast) 2953 (s), 2929(s), 2857 (m), 1726 (s), 1171 (s), 836 (s), cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.15 (ddt, 1 H, *J* = 152.1, 11.5, 7.5 Hz, $^{13}C\underline{H}{=}^{13}CHCO$), 5.71 (dd, 1 H, *J* = 164.1, 11.5 Hz, $^{13}CH{=}^{13}C\underline{H}CO$), 3.74 (m, 1 H, $C\underline{H}(OSi)$), 3.63 (s, 3 H, $OC\underline{H}_3$), 2.60 (m, 2 H, $C\underline{H}_2{}^{13}CH$), 1.65-1.25 (m, 4 H, $CH(OSi)C\underline{H}_2C\underline{H}_2$), 1.06 (d, 3 H, *J* = 6.2 Hz, $C\underline{H}_3CH(OSi)$), 0.82 (s, 9 H, $(C\underline{H}_3)_3C$), -0.02 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 166.80 (d, *J* = 75.5

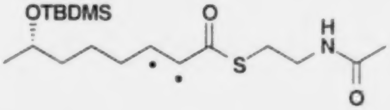
Hz, $\underline{C}$O), 150.48 (d, *J* = 69.4 Hz, enriched, $^{13}\underline{C}$H=^{13}CHCO), 119.43 (d, *J* = 69.4 Hz, enriched, ^{13}CH=$^{13}\underline{C}$HCO), 68.19 ($\underline{C}$H(OSi)), 50.83 (O$\underline{C}$H$_3$), 39.11 (CH(OSi)$\underline{C}$H$_2$), 28.80 (d, *J* = 40.6 Hz, $\underline{C}$H$_2$^{13}CH), 25.82 (($\underline{C}$H$_3$)$_3$C), 24.40 (CH(OSi)CH$_2$$\underline{C}H_2$), 23.73 ($\underline{C}H_3$CH(OSi)), 18.02 ((CH$_3$)$_3$$\underline{C}$), -4.42 and -4.84 (($\underline{C}H_3$)$_2$Si); MS (CI, NH$_3$) *m/z* (relative intensity) 306 (MNH$_4^+$, 85), 289 (MH$^+$, 53); HRMS (EI) *m/z* (relative intensity) 273.1798 (3), 231.1322 (100).



Methyl (*S*)-[2,3-^{13}C$_2$]-7-(*tert*-Butyldimethylsilyloxy)octanoate (**33**) The procedure employed for the conversion of **20a** to **21b** was used. Thus, unsaturated ester **32** as a mixture of *E* and *Z* isomers (517 mg, 1.79 mmol) was hydrogenated in the presence of 10% Pd/C (55 mg) to afford **33** (488 mg, 94%). $[\alpha]_D$ +8.93° (*c* 1.49, CHCl$_3$); IR (CHCl$_3$ cast) 2930 (s), 1743 (s), 1255 (m), 1051 (m), 836 (s), 774 (m) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 3.76 (m, 1 H, C$\underline{H}$(OSi)), 3.66 (s, 3 H, OC$\underline{H}_3$), 2.30 (dm, 2 H, *J* = 127.6 Hz, ^{13}C$\underline{H}_2$CO), 1.63 (dm, 2 H, *J* = 136.0 Hz, ^{13}C$\underline{H}_2$^{13}CH$_2$CO), 1.50-1.20 (m, 6 H, CH(OSi)C$\underline{H}_2$C$\underline{H}_2$C$\underline{H}_2$), 1.10 (d, 3 H, *J* = 6.0 Hz, C$\underline{H}_3$CH(OSi)), 0.89 (s, 9 H, (C$\underline{H}_3$)$_3$C), 0.04 (s, 6 H, (C$\underline{H}_3$)$_2$Si); ^{13}C NMR (100 MHz, CDCl$_3$) δ 174.03 (d, *J* = 56.6 Hz, $\underline{C}$O), 68.47 ($\underline{C}$H(OSi)), 51.37 (O$\underline{C}$H$_3$), 39.50 (CH(OSi)$\underline{C}$H$_2$), 34.03 (d, *J* = 33.9 Hz, enriched, $^{13}\underline{C}$H$_2$CO), 29.20 (d, *J* = 34.6 Hz, $\underline{C}$H$_2$^{13}CH$_2$), 25.87 (($\underline{C}$H$_3$)$_3$C), 25.34 (CH(OSi)CH$_2$$\underline{C}H_2$), 24.93 (d, *J* = 33.9 Hz, enriched, $^{13}\underline{C}$H$_2$^{13}CH$_2$CO), 23.78 ($\underline{C}$H$_3$CH(OSi)), 18.11 ((CH$_3$)$_3$$\underline{C}$), -4.43 and -4.75 (($\underline{C}H_3$)$_2$Si); MS (CI, NH$_3$) *m/z* (relative intensity) 308 (MNH$_4^+$, 4), 291 (MH$^+$, 64); HRMS (EI) *m/z* (relative intensity) 275.1959 (4), 259.2002 (10) 233.1483 (78), 201.1220 (100).

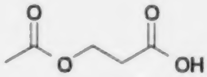


(*S*)-[2,3-$^{13}C_2$]-7-(*tert*-Butyldimethylsilyloxy)octanoic Acid (34) The procedure employed for the conversion of **21b** to **22a** was used. Thus, **33** (425 mg, 1.46 mmol) was hydrolyzed with 1N NaOH (2.8 mL) to give **34** (399 mg, 99%). $[\alpha]_D$ +11.2° (*c* 2.33, $CHCl_3$); IR ($CHCl_3$ cast) 3300-2600 (br m), 2930 (s), 1711 (s), 1255 (m), 1051 (m), 836 (s), 774 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 11.32 (br s, 1 H, $CCO\underline{H}$), 3.74 (m, 1 H, $C\underline{H}(OSi)$), 2.30 (dm, 2 H, J = 126.0 Hz, $^{13}C\underline{H}_2CO$), 1.61 (dm, 2 H, J = 135.0 Hz, $^{13}C\underline{H}_2{}^{13}CH_2CO$), 1.40-1.20 (m, 6 H, $CH(OSi)C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 1.09 (d, 3 H, J = 6.0 Hz, $C\underline{H}_3CH(OSi)$), 0.86 (s, 9 H, $(C\underline{H}_3)_3C$), 0.02 (s, 6 H, $(C\underline{H}_3)_2Si$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.13 (d, J = 55.1 Hz, $\underline{C}OOH$), 68.52 ($\underline{C}H(OSi)$), 39.46 ($CH(OSi)\underline{C}H_2$), 34.03 (d, J = 34.4 Hz, enriched, $^{13}\underline{C}H_2CO$), 29.13 (d, J = 34.6 Hz, $\underline{C}H_2{}^{13}CH_2$), 25.90 ($(\underline{C}H_3)_3C$), 25.38 ($CH(OSi)CH_2\underline{C}H_2$), 24.66 (d, J = 34.4 Hz, enriched, $^{13}\underline{C}H_2{}^{13}CH_2CO$), 23.80 ($\underline{C}H_3CH(OSi)$), 18.14 ($(CH_3)_3\underline{C}$), -4.40 and -4.72 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) *m/z* (relative intensity) 294 (MNH_4^+, 6), 277 (MH^+, 100); HRMS (EI) *m/z* (relative intensity) 243.1694 (4), 219.1327 (14) 201.1224 (100).

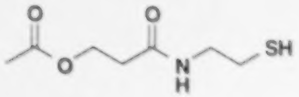


NAC (*S*)-[2,3-$^{13}C_2$]-7-(*tert*-Butyldimethylsilyloxy)octanoate (35) The procedure employed for the preparation of **7a** was used. Thus, acid **34** (354 mg, 1.28 mmol) was coupled with *N*-acetylcysteamine (168 mg, 1.41 mmol) in the presence of DCC (291 mg, 1.41mmol) and 4-dimethylaminopyridine (8 mg) to afford NAC ester **35** (430 mg, 89%). $[\alpha]_D$ +6.40° (*c* 1.75, $CHCl_3$); IR ($CHCl_3$ cast) 3286 (br m), 2930 (s), 2857 (m), 1693 (s), 1654 (s), 1554 (m), 836 (s), 774 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.95 (br s, 1 H, $N\underline{H}$), 3.77 (m, 1 H, $C\underline{H}(OSi)$), 3.43 (q, 2 H, J = 6.3 Hz, $NHC\underline{H}_2$), 3.30 (t, 2 H, J = 6.3 Hz, $C\underline{H}_2S$), 2.57 (dm, 2 H, J = 129.1 Hz, $^{13}C\underline{H}_2CO$), 1.97 (s, 3 H, $COCH_3$), 1.70 (dm, 2 H, J =

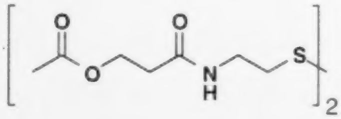
133.6 Hz, $^{13}\mathrm{C\underline{H}_2{}^{13}CH_2CO}$), 1.42-1.20 (m, 6 H, $\mathrm{CH(OSi)C\underline{H}_2C\underline{H}_2C\underline{H}_2}$), 1.11 (d, 3 H, J = 6.0 Hz, $\mathrm{C\underline{H}_3CH(OSi)}$), 0.88 (s, 9 H, $\mathrm{(C\underline{H}_3)_3C}$), 0.05 (s, 3 H, $\mathrm{C\underline{H}_3SiCH_3}$), 0.04 (s, 3 H, $\mathrm{CH_3SiC\underline{H}_3}$); ^{13}C NMR (100 MHz, $\mathrm{CDCl_3}$) δ 200.07 (d, J = 46.0 Hz, $\mathrm{\underline{C}OS}$), 170.19 ($\mathrm{\underline{C}OCH_3}$), 68.42 ($\mathrm{\underline{C}H(OSi)}$), 44.02 (d, J = 33.3 Hz, enriched, $^{13}\mathrm{\underline{C}H_2CO}$), 39.71 ($\mathrm{NH\underline{C}H_2}$), 39.38 ($\mathrm{CH(OSi)\underline{C}H_2}$), 28.98 (d, J = 34.6 Hz, $\mathrm{\underline{C}H_2{}^{13}CH_2}$), 28.41 ($\mathrm{\underline{C}H_2S}$), 25.59 (d, J = 33.3 Hz, enriched, $^{13}\mathrm{\underline{C}H_2{}^{13}CH_2CO}$), signals for ($\mathrm{(\underline{C}H_3)_3C}$) and ($\mathrm{CH(OSi)CH_2\underline{C}H_2}$) were obscured by this enriched signal, 23.76 ($\mathrm{\underline{C}H_3CH(OSi)}$), 23.15 ($\mathrm{CO\underline{C}H_3}$), 18.09 ($\mathrm{(CH_3)_3\underline{C}}$), -4.42 and -4.75 ($\mathrm{(\underline{C}H_3)_2Si}$); MS (CI, $\mathrm{NH_3}$) *m/z* (relative intensity) 395 ($\mathrm{MNH_4^+}$, 7), 378 ($\mathrm{MH^+}$, 100); HRMS (EI) *m/z* (relative intensity) 362.2120 (4), 320.1626 (100).



3-Acetoxypropionic acid (**37**) To a stirring suspension of sodium acetate (20.5 g, 250 mmol) in acetic acid (150 mL) was added dropwise a solution of propiolactone (3.5 mL, Sigma grade II, >90%, 50 mmol) in $\mathrm{CH_2Cl_2}$ (10 mL). The mixture was then stirred at room temperature for 30 min. Acetic acid was removed *in vacuo* at 60 °C, the solid residue was dissolved with water (200 mL), acidified with conc. HCl to pH 2, extracted with ether (3 x 200 mL), and the combined extracts were then dried ($\mathrm{Na_2SO_4}$). After removal of solvent the residue was purified by vacuum distillation to afford **37** (5.60 g, 85%). Bp 102-104 °C at 8 mm Hg. IR ($\mathrm{CHCl_3}$ cast) 3500-2500 (br m), 2973 (m), 1743 (s), 1719 (s), 1241 (s), 1043 (s) $\mathrm{cm^{-1}}$; ^{1}H NMR (300 MHz, $\mathrm{CDCl_3}$) δ 10.42 (br s, 1 H, $\mathrm{COO\underline{H}}$), 4.29 (t, 2 H, J = 6.4 Hz, $\mathrm{OC\underline{H}_2}$), 2.66 (t, 2 H, J = 6.4 Hz, $\mathrm{C\underline{H}_2COOH}$), 2.02 (s, 3 H, $\mathrm{C\underline{H}_3CO}$); ^{13}C NMR (75 MHz, $\mathrm{CDCl_3}$) δ 176.05 ($\mathrm{\underline{C}OOH}$), 171.11 ($\mathrm{CH_3\underline{C}O}$), 59.47 ($\mathrm{O\underline{C}H_2}$), 33.34 ($\mathrm{\underline{C}H_2COOH}$), 20.55 ($\mathrm{\underline{C}H_3CO}$); MS (CI, $\mathrm{NH_3}$) *m/z* (relative intensity) 150 ($\mathrm{MNH_4^+}$, 100), 133 ($\mathrm{MH^+}$, 7); MS (EI) *m/z* (relative intensity) calcd for $\mathrm{C_5H_7O_3}$ 115.0395, found 115.0389 ($\mathrm{M^+}$ -OH, 6), 86.0237 (85), 73.0265 (100); Anal. Calcd for $\mathrm{C_5H_8O_4}$: C, 45.45; H, 6.10. Found: C, 45.12; H, 6.18.

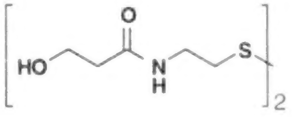


***N*-(3-Acetoxypropionyl)cysteamine** (**38**) The method for the preparation of **39** was used. Thus cysteamine hydrochloride (455 mg, 4 mmol) afforded **38** (390 mg, 51%). IR ($\mathrm{CH_2Cl_2}$ cast) 3304 (br m), 2934 (m), 2560 (w), 1739 (s), 1652 (s), 1548 (s), 1237 (s) cm^{-1}; ^{1}H NMR (300 MHz, $\mathrm{CDCl_3}$) δ 6.25 (br s, 1 H, $\mathrm{N\underline{H}}$), 4.35 (t, 2 H, J = 6.2 Hz, $\mathrm{OC\underline{H}_2}$), 3.44 (q, 2 H, J = 6.2 Hz, $\mathrm{NHC\underline{H}_2}$), 2.67 (dt, 2 H, J = 8.5, 6.3 Hz, $\mathrm{C\underline{H}_2SH}$), 2.53 (t, 2 H, J = 6.2 Hz, $\mathrm{C\underline{H}_2CO}$), 2.05 (s, 3 H, $\mathrm{C\underline{H}_3CO}$), 1.37 (t, 1 H, J = 8.5 Hz, $\mathrm{S\underline{H}}$); ^{13}C NMR (75 MHz, $\mathrm{CDCl_3}$) δ 170.81 ($\mathrm{CH_3\underline{C}O}$), 169.90 ($\mathrm{CH_2\underline{C}O}$), 60.49 ($\mathrm{O\underline{C}H_2}$), 42.31 ($\mathrm{NH\underline{C}H_2}$), 35.79 ($\mathrm{\underline{C}H_2CO}$), 24.52 ($\mathrm{\underline{C}H_2SH}$), 20.88 ($\mathrm{\underline{C}H_3CO}$); MS (CI, $\mathrm{NH_3}$) *m/z* (relative intensity) 209 ($\mathrm{MNH_4^+}$, 14), 192 ($\mathrm{MH^+}$, 100); HRMS (EI) calcd for $\mathrm{C_7H_{13}NO_3S}$ 191.0616, found 191.0616.

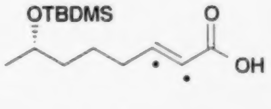


***N,N'*-Bis(3-acetoxypropionyl)cystamine** (**39**) 3-Acetoxypropionic acid (555 mg, 4.2 mmol), cystamine hydrochloride (450 mg, 2 mmol), 1-hydroxybenzotriazole (HOBT) (568 mg, 4.2 mmol) and triethylamine (0.59 mL, 4.2 mmol) were dissolved in $\mathrm{CH_2Cl_2}$ (20 mL). A solution of DCC (867 mg, 4.2 mmol) in $\mathrm{CH_2Cl_2}$ (10 mL) was added at 0 °C. The mixture was then warmed to room temperature and stirred for 8 h. The white precipitate was removed by filtration and washed with $\mathrm{CH_2Cl_2}$, the filtrate was washed successively twice with 0.5 N HCl, once with 5% $\mathrm{NaHCO_3}$, and once with brine, then dried ($\mathrm{Na_2SO_4}$). After concentration *in vacuo*, the residue was purified by $\mathrm{SiO_2}$ column chromatography (EtOAc-10% $\mathrm{CH_3OH}$ in EtOAc) to afford **39** (373 mg, 49%) as a solid. IR (KBr) 3252 (m), 1737 (s), 1658 (m), 1559 (m), 1251 (s), 1046 (m) cm^{-1}; ^{1}H NMR (300 MHz, $\mathrm{CDCl_3}$)

δ 6.63 (br s, 1 H, $N\underline{H}$), 4.36 (t, 2 H, J = 6.3 Hz, $OC\underline{H}_2$), 3.59 (q, 2 H, J = 6.3 Hz, $NHC\underline{H}_2$), 2.85 (t, 2 H, J = 6.5 Hz, $C\underline{H}_2S$), 2.56 (t, 2 H, J = 6.3 Hz, $C\underline{H}_2CO$), 2.05 (s, 3 H, $C\underline{H}_3CO$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.84 ($CH_3\underline{C}O$), 170.36 ($CH_2\underline{C}O$), 60.48 ($O\underline{C}H_2$), 38.43 ($NH\underline{C}H_2$), 37.68 ($\underline{C}H_2S$), 35.66 ($\underline{C}H_2CO$), 20.90 ($\underline{C}H_3CO$); MS (CI, NH_3) *m/z* (relative intensity) 398 (MNH_4^+, 15), 381 (MH^+, 39); HRMS (EI) *m/z* (relative intensity) 249.0487 (2), 223.0336 (2), 191.0613 (49), 98.0606 (100); Anal. Calcd for $C_{14}H_{24}N_2O_6S_2$: C, 44.20; H, 6.36; N, 7.36; S, 16.85. Found: C, 43.98; H, 6.47; N, 7.26; S, 16.80.

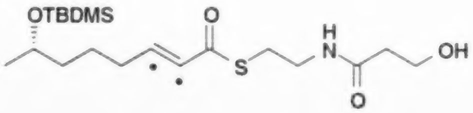


***N,N'*-Bis(3-hydroxypropionyl)cystamine** (**40**) To a solution of **39** (440 mg, 1.16 mmol) in CH_3OH (15 mL) was added LiOH solution (194 mg of $LiOH{\cdot}H_2O$ in 5 mL of H_2O, 4.62 mmol) at 0 °C. The mixture was then stirred at room temperature overnight. After the removal of MeOH the residue was acidified to pH 1, extracted with CH_2Cl_2, and dried (Na_2SO_4). Concentration gave the crude **40** (398 mg), which was used for the next step directly. IR (neat) 3285 (br, s), 1649 (s), 1554 (m), 1044 (m) cm^{-1}; ^{1}H NMR (360 MHz, $CD_3CN + CD_3OD$) δ 3.71 (t, 2 H, J = 6.1 Hz, $HOC\underline{H}_2$), 3.43 (t, 2H, J = 6.8Hz, $NHC\underline{H}_2$), 2.87 (t, 2 H, J = 6.8 Hz, $C\underline{H}_2S$), 2.34 (t, 2 H, J = 6.1 Hz, $C\underline{H}_2CO$); ^{13}C NMR (75 MHz, $CD_3CN + CD_3OD$) δ 173.86 ($CH_2\underline{C}O$), 58.89 ($HO\underline{C}H_2$), 39.49, 39.05, 38.21.



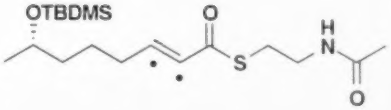
(*S*)-*E*-[2,3-$^{13}C_2$]-7-(*tert*-Butyldimethylsilyloxy)oct-2-enoic Acid (**41**) The procedure for the conversion of **39** to **40** was followed. Thus, ester **32** (*E*-isomer only, 400 mg, 1.38 mmol) was hydrolyzed with 0.25 M LiOH (11 mL) in methanol (25 mL) to give **41**(314 mg, 83%). IR ($CHCl_3$ cast) 2956 (s), 2930(s), 2858 (m), 1695 (s), 836 (s), 774 (m) cm^{-1};

^{1}H NMR (300 MHz, CDCl$_3$) δ 7.08 (ddt, 1 H, *J* = 154.6, 15.6, 7.1 Hz, ^{13}C$\underline{\mathrm{H}}$=^{13}CHCO), 5.83 (dd, 1 H, *J* = 162.8, 15.6 Hz, ^{13}CH=^{13}C$\underline{\mathrm{H}}$CO), 3.80 (m, 1 H, C$\underline{\mathrm{H}}$(OSi)), 2.24 (m, 2 H, C$\underline{\mathrm{H}}_2$^{13}CH), 1.70-1.30 (m, 4 H, CH(OSi)C$\underline{\mathrm{H}}_2$C$\underline{\mathrm{H}}_2$), 1.13 (d, 3 H, *J* = 6.1 Hz, C$\underline{\mathrm{H}}_3$CH(OSi)), 0.89 (s, 9 H, (C$\underline{\mathrm{H}}_3$)$_3$C), 0.05 (s, 6 H, (C$\underline{\mathrm{H}}_3$)$_2$Si); ^{13}C NMR (75 MHz, CDCl$_3$) δ 171.66 (d, *J* = 72.1 Hz, $\underline{\mathrm{C}}$O), 152.17 (d, *J* = 69.8 Hz, enriched, ^{13}$\underline{\mathrm{C}}$H=^{13}CHCO), 120.60 (d, *J* = 69.8 Hz, enriched, ^{13}CH=^{13}$\underline{\mathrm{C}}$HCO), 68.20 ($\underline{\mathrm{C}}$H(OSi)), 39.04 (CH(OSi)$\underline{\mathrm{C}}$H$_2$), 32.55 (d, *J* = 43.2 Hz, $\underline{\mathrm{C}}$H$_2$^{13}CH=), 25.87 (($\underline{\mathrm{C}}$H$_3$)$_3$C), 24.03 (CH(OSi)CH$_2$$\underline{\mathrm{C}}H_2$), 23.80 ($\underline{\mathrm{C}}H_3$CH(OSi)), 18.12 ((CH$_3$)$_3$$\underline{\mathrm{C}}$), -4.37 and -4.74 (($\underline{\mathrm{C}}H_3$)$_2$Si); MS (CI, NH$_3$) *m/z* (relative intensity) 292 (MNH$_4^+$, 16), 275 (MH$^+$, 32); HRMS (EI) *m/z* (relative intensity) 259.1650 (1), 217.1175 (21), 199.1074 (100).

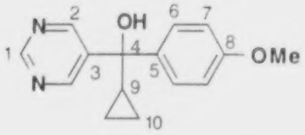


***N*-(3-Hydroxypropionyl)cysteaminyl (*S*)-*E*-[2,3-^{13}C$_2$]-7-(*tert*-Butyldimethylsilyloxy)oct-2-enoate (42)** Acid **28** (66 mg, 0.44 mmol), *N*-acetylcysteamine (66 mg, 0.48 mmol), HOBT (60 mg, 0.48 mmol) and 4-dimethylaminopyridine (10 mg) in CH$_2$Cl$_2$ were stirred with molecular sieves (4 Å, 2 g) for ca. 10 min. DCC (91 mg, 0.48) in CH$_2$Cl$_2$ (2 mL) was then added, and the mixture was stirred at room temperature overnight. The molecular sieves and precipitate formed were removed by filtration. The filtrate was then concentrated and purified with SiO$_2$ chromatography (20:1 CHCl$_3$-CH$_3$OH) to give **42** (43 mg, 27%). IR (CH$_2$Cl$_2$ cast) 3304 (br m), 2928 (s), 2856 (m), 1654 (s), 1549 (m), 1047 (m), 838 (m), 774 (m) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 6.90 (ddt, 1 H, *J* = 153.7, 15.6, 7.3 Hz, ^{13}C$\underline{\mathrm{H}}$=^{13}CHCO), 6.50 (br s, 1 H, N$\underline{\mathrm{H}}$), 6.11 (dd, 1 H, *J* = 161.1, 15.6 Hz, ^{13}C$\underline{\mathrm{H}}$CO), 3.85 (t, 2 H, *J* = 5.5 Hz, C$\underline{\mathrm{H}}_2$OH), 3.78 (m, 1 H, C$\underline{\mathrm{H}}$(OSi)), 3.46 (q, 3 H, *J* = 6.4 Hz, C$\underline{\mathrm{H}}_2$NH + O$\underline{\mathrm{H}}$), 3.09 (t, 2 H, *J* = 6.4 Hz, SC$\underline{\mathrm{H}}_2$), 2.41 (t, 2 H, *J* = 5.5 Hz, COC$\underline{\mathrm{H}}_2$), 2.19 (m, 2 H, C$\underline{\mathrm{H}}_2$^{13}CH), 1.70-1.20 (m, 4 H,

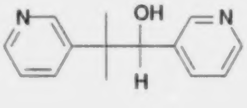
$CH(OSi)C\underline{H}_2C\underline{H}_2$), 1.11 (d, 3 H, *J* = 6.1 Hz, $C\underline{H}_3CH(OSi)$), 0.87 (s, 9 H, $(C\underline{H}_3)_3C$), 0.04 (s, 3 H, $C\underline{H}_3SiCH_3$), 0.03 (s, 3 H, $CH_3SiC\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.28 (d, *J* = 60.8 Hz, $\underline{C}OS$), 172.65 ($\underline{C}OCH_2$), 146.53 (d, *J* = 69.4 Hz, enriched, $^{13}\underline{C}H=^{13}CHCO$), 128.26 (d, *J* = 69.4 Hz, enriched, $^{13}\underline{C}HCO$), 68.14 ($\underline{C}H(OSi)$), 58.76 ($\underline{C}H_2OH$), 39.52 ($\underline{C}H_2NH$), 38.98 ($CH(OSi)\underline{C}H_2$), 38.03 ($CO\underline{C}H_2$), 32.20 (d, *J* = 41.0 Hz, $CH_2{}^{13}CH$), 28.11 ($S\underline{C}H_2$), 25.83 ($(\underline{C}H_3)_3C$) 23.98 ($\underline{C}H_2CH_2{}^{13}CH$), 23.73 ($\underline{C}H_3CH(OSi)$), 18.10 ($(CH_3)_3\underline{C}$), -4.43 and -4.78 ($(CH_3)_2Si$); MS (CI, NH_3) *m/z* (relative intensity) 406 (MH^+, 100); HRMS (EI) *m/z* (relative intensity) 348.1579 (51), 206.0671 (67), 116.0712 (100).



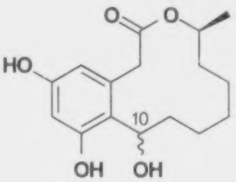
NAC (*S*)-[2,3-$^{13}C_2$]-7-(*tert*-Butyldimethylsilyloxy)oct-2-enoate (**43**) The procedure for the preparation of **7a** was adopted. Thus, **42** (287 mg, 1.05 mmol) was coupled with *N*-acetylcysteamine (142 mg, 1.15 mmol) in the presence of DCC (246 mg, 1.15 mmol) and 4-dimethylaminopyridine (20 mg) to give **43** (125 mg, 32%). IR ($CHCl_3$ cast) 3287 (br m), 2929 (s), 2857 (m), 1657 (s), 1046 (m), 836 (s), 775 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.87 (ddt, 1 H, *J* = 153.4, 15.6, 7.5 Hz, $^{13}C\underline{H}=^{13}CHCO$), 6.32 (br s, 1 H, $N\underline{H}$), 6.08 (dd, 1 H, *J* = 160.9, 15.6 Hz, $^{13}C\underline{H}CO$), 3.75 (m, 1 H, $C\underline{H}(OSi)$), 3.41 (q, 2 H, *J* = 6.2 Hz, $C\underline{H}_2NH$), 3.05 (t, 2 H, *J* = 6.4 Hz, $SC\underline{H}_2$), 2.17 (m, 2 H, $C\underline{H}_2{}^{13}CH$), 1.93 (s, 3 H, $C\underline{H}_3CO$), 1.58-1.30 (m, 4 H, $CH(OSi)C\underline{H}_2C\underline{H}_2$), 1.08 (d, 3 H, *J* = 6.1 Hz, $C\underline{H}_3CH(OSi)$), 0.84 (s, 9 H, $(C\underline{H}_3)_3C$), 0.00 (s, 6 H, $Si(C\underline{H}_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.12 (d, *J* = 61.9 Hz, $\underline{C}OS$), 170.31 ($\underline{C}OCH_3$), 146.26 (d, *J* = 69.4 Hz, enriched, $^{13}\underline{C}H=^{13}CHCO$), 128.26 (d, *J* = 69.4 Hz, enriched, $^{13}\underline{C}HCO$), 68.05 ($\underline{C}H(OSi)$), 39.63 ($\underline{C}H_2NH$), 38.92 ($CH(OSi)\underline{C}H_2$), 32.12 (d, *J* = 41.3 Hz, $CH_2{}^{13}CH$), 28.11 ($S\underline{C}H_2$), 25.77 ($(\underline{C}H_3)_3C$), 23.92 ($\underline{C}H_2CH_2{}^{13}CH$), 23.67 ($\underline{C}H_3CH(OSi)$), 23.02 ($\underline{C}H_3CO$), 17.98 ($(CH_3)_3\underline{C}$), -4.48 and -4.83 ($(CH_3)_2Si$); MS (CI, NH_3) *m/z* (relative intensity) 393 (MNH_4^+, 28), 376 (MH^+, 86); HRMS (EI) *m/z* (relative intensity) 360.1934 (5), 318.1471 (100).



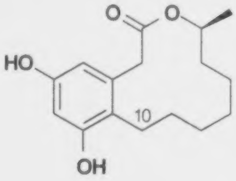
Acymidol (44) The procedure of Taylor *et al.*[47] was repeated. To the slurry of 5-bromopyrimidine (19.87 g, 0.125 mole) and cyclopropyl 4-methoxyphenyl ketone (22.03 g, 0.125 mole) in dry THF (350 mL) cooled to -95 °C, was added 1.6 M *n* butyllithium in hexane (78 mL) over a period of 1 h. The solution was carefully warmed to room temperature and stirred overnight before being quenched with H_2O (80 mL) at -20 °C. The aqueous layer was extracted once with toluene, the combined organic layers were then washed once with 0.5 N HCl, three times with H_2O, and dried (Na_2SO_4). Evaporation of solvents gave 31.34 g solid, which was recrystallized from Et_2O to give pure **44** (21.0 g, 66%). Mp 110-111 °C (lit.[47] 111 °C) IR ($CHCl_3$ cast) 3245 (br m), 1565 (m), 1510 (s), 1405 (s), 1255 (s), 1180 (s), 1015 (m), 830 (m) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.95 (s, 1 H, H-1), 8.68 (s, 2 H, H-2), 7.36 (m, 2 H, H-6), 6.85 (m, 2 H, H-7), 3.78 (s, 3 H, OC$\underline{H}_3$), 1.52 (m, 1 H, H-9), 0.73 (m, 1 H, H-10), 0.60-0.43 (m, 3 H, H-10); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.20 (C-3), 156.22 (C-1), 154.99 (C-2), 144.14 (C-8), 137.18 (C-5), 128.29 (C-6), 113.77 (C-7), 74.24 (C-4), 55.23 (OCH_3), 21.48 (C-9), 2.53 and 0.92 (2 x C-10); MS (EI) *m/z* (relative intensity) calcd 256 (M^+, 9), 228 (100), 215 (27). Anal. calcd for $C_{15}H_{16}N_2O_2$: C, 70.13; H, 6.34; N, 10.89. Found C, 70.29; H, 6.29; N, 10.93.



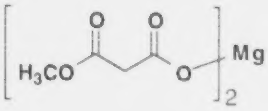
2-Methyl-1,2-di-(3-pyridyl)-1-propanol (47) Extracts (523 mg) from the culture of *A. cinerariae* (4 x 100 mL) treated with metyropone, were separated with SiO_2 chromatography (3:1 $CHCl_3$-EtOAc, EtOAc, 5:1 EtOAc-CH_3OH). Alcohol **47** (111 mg) was obtained in addition to dehydrocurvularin, 8-hydroxycurvularin and metyropone. $[\alpha]_D$ -31.2° (*c* 1.82, CH_2Cl_2); IR ($CHCl_3$ cast) 3170 (br s), 2965 (s), 1580 (m), 1480 (m), 1415 (s), 1065 (m), 1025 (m), 750 (m), 715 (m) cm^{-1}; ^{1}H NMR (400 MHz, acetone-d_6) δ 8.52 (br s, 1 H, Ar-H), 8.36 (br s, 2 H, Ar-H), 8.20 (br s, 1 H, Ar-H), 7.67 (dt, 1 H, *J* = 8.0, 1.9 Hz, Ar-H), 7.34 (dt, 1 H, *J* = 7.8, 1.9 Hz, Ar-H), 7.23 (AB, 1 H, Ar-H), 7.15 (dd, 1 H, *J* = 7.8, 4.7 Hz, Ar-H), 4.85 (s, 1 H, C$\underline{\text{H}}$OH), 2.95 (br s, 1 H, O$\underline{\text{H}}$), 1.36 (s, 6 H, 2 x CH_3); ^{13}C NMR (100 MHz, acetone-d_6) δ 150.06, 149.78, 149.10, 147.96, 142.22, 138.47, 135.82, 135.54, 123.31, 123.05, 79.41, 42.74, 25.00, 24.21; MS (CI, NH_3) *m/z* (relative intensity) 229 (MH^+, 100); MS (EI) *m/z* (relative intensity) 121.0890 (100), 109.0529 (15), 92.0503 (22).



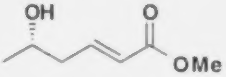
10-Hydroxy-10-dihydrocurvularin (48) To a solution of **2** (103 mg, 0.352 mmol) in 95% ethanol (20 mL) cooled to -15 °C was slowly added a solution of $NaBH_4$ (20 mg, 0.53 mmol) in 95% ethanol (5 mL). The mixture was stirred at 0 °C for 1 h before a saturated solution of NH_4Cl was added. Ethanol was then removed *in vacuo*, and the residue was extracted four times with $CHCl_3$, then dried (Na_2SO_4). Flash chromatography on SiO_2 (2:1 hexane-EtOAc) gave two fractions, which were identified as the two isomers of **48**, the less polar isomer (35.5 mg, 35%) and the more polar isomer (38.8 mg, 38%). For the less polar isomer: IR (KBr) 3452 (s), 3393 (s), 3271 (br m), 2940 (m), 1700 (s), 1655 (m), 1460 (m), 1318 (s), 1135 (s) cm^{-1}; ^{1}H NMR (300 MHz, acetone-d_6) δ 8.86 (br s, 1 H, Ar-O$\underline{\text{H}}$), 8.11 (br s, 1 H, Ar-O$\underline{\text{H}}$), 6.28 (d, 1 H, *J* = 2.8 Hz, Ar-$\underline{\text{H}}$), 6.22 (d, 1 H, *J* = 2.8 Hz, Ar-$\underline{\text{H}}$), 5.32 (br s, 1 H, O$\underline{\text{H}}$), 5.14-5.04 (m, 2 H, H-4, H-10), 3.65 (d, 2 H, 2 x H-1), 3.38 (br d, 1 H, *J* = 17.0 Hz, 1 x H-1), 1.91-1.09 (m, 10 H, 2 x H-5, 2 x H-6, 2 x H-7, 2 x H-8, 2 x H-9), 1.19 (d, 3 H, *J* = 7.0 Hz, $C\underline{H}_3$); ^{13}C NMR (50 MHz, acetone-d_6) δ 173.53 (C-2), 158.82 (C-11), 158.16 (C-13), 134.05 (C-15), 121.21 (C-16), 111.78 (C-14), 104.13 (C-12), 78.81 (C-10), 69.56 (C-4), 40.29 (C-1), 34.88, 32.96, 27.57, 24.54, 23.53, 20.53 ($\underline{C}H_3$); HRMS (EI) *m/z* (relative intensity) calcd for $C_{16}H_{22}O_5$ 294.1467, found 294.1486 (M^+, 13), 292 (45), 205 (3), 195 (100), 167 (94), 150 (65).



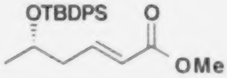
10-Dihydrocurvularin (**49**) Under an atmosphere of H_2 a solution of **48** (10 mg, 0.034 mmol) and 10% palladium over carbon (8 mg) in ethyl acetate (2 mL) was stirred for 5 h. After filtration and evaporation of solvent the crude material was purified with column chromatography (3:2 $CHCl_3$-EtOAc) to afford **49** (7.0 mg, 70%). IR (KBr) 3405 (br s), 2929 (m), 1702 (s), 1629 (m), 1600 (m), 1318 (m), 1208 (m), 1134 (s) cm^{-1}; ^1H NMR (400 MHz, acetone-d_6) δ 7.99 (s, 1 H, Ar-O$\underline{H}$), 7.89 (s, 1 H, Ar-O$\underline{H}$), 6.31 (d, 1 H, *J* = 2.4 Hz, Ar-$\underline{H}$), 6.28 (d, 1 H, *J* = 2.4 Hz, Ar-$\underline{H}$), 5.07 (m, 1 H, H-4), 3.69 (d, 1 H, *J* = 15.5 Hz, H-1), 3.36 (d, 1 H, *J* = 15.5 Hz, H-1), 2.54 (m, 2 H, 2 x H-10), 1.84-1.69 (m, 2 H, 2 x H-5), 1.66-1.49 (m, 2 H, 2 x H-9), 1.14-1.10 (m, 4 H, 2 x H-6, 2 x H-7, 2 x H-8), 1.18 (d, 3 H, *J* = 6.3 Hz, $C\underline{H}_3$); ^{13}C NMR (100 MHz, acetone-d_6) δ 171.99 (C-2), 157.21 (C-11), 156.39 (C-13), 135.80 (C-15), 120.03 (C-16), 111.21 (C-14), 102.45 (C-12), 73.32 (C-4), 40.29 (C-1), 33.95 (C-5), 27.39, 27.34, 26.91, 25.36, 25.12, 20.86 ($\underline{C}H_3$); MS (EI) *m/z* (relative intensity) calcd for $C_{16}H_{22}O_4$ 278.1518, found 278.1514 (M^+, 71), 219.1383 (8), 207.0657 (20), 181.0500 (100).



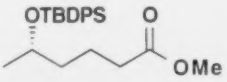
Magnesium Methyl Malonate (52) The procedure of Brooks *et al.*[48b] was used. To a solution of monomethyl hydrogen malonate (1.096 g, 9.29 mmol) in THF (25 mL) was added magnesium ethoxide (0.53 g, 4.64 mmol) in several portions. The mixture was then stirred at room temperature for 4 h. After removal of solvent *in vacuo*, **52** was obtained as a white solid (1.07 g, 89%). ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 3.62 (s, 3 H, OC$\underline{H}_3$), 3.57 (s, 2 H, CH$_2$); ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 170.93 ($\underline{C}O_2$Mg), 169.42 ($\underline{C}O_2CH_3$), 51.35 (O$\underline{C}H_3$), 44.33 ($\underline{C}H_2$).



Methyl (*S*)-5-Hydroxyhex-2-enoate (54) The procedure employed for the conversion of **23a** to **8a** was used. Thus, silyl ether **20a** (468 mg, 1.81 mmol) was deprotected with boron trifluoride diethyl etherate (1.5 mL, 12 mmol) to give alcohol **54** (247 mg, 95%). IR (CHCl$_3$ cast) 3435 (br m), 1724 (s), 1658 (m), 1273 (m), 1172 (m) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 6.97 (dt, 1 H, *J* = 15.6, 7.5 Hz, C$\underline{H}$=CHCO), 5.90 (dt, 1 H, *J* = 15.6, 1.4 Hz, CH=C$\underline{H}$CO), 3.96 (m, 1 H, C$\underline{H}$(OSi)), 3.72 (s, 3 H, OC$\underline{H}_3$), 2.36 (m, 2 H, C$\underline{H}_2$CH), 1.94 (br s, 1 H, O$\underline{H}$), 1.23 (d, 3 H, *J* = 6.2 Hz, C$\underline{H}_3$CH(OSi)); ^{13}C NMR (75 MHz, CDCl$_3$) δ 166.75 ($\underline{C}$O), 145.35 ($\underline{C}$H=CHCO), 123.41 (CH=$\underline{C}$HCO), 66.67 ($\underline{C}$H(OSi)), 51.45 (O$\underline{C}H_3$), 41.79 (CH$_2$CH), 23.17 ($\underline{C}H_3$CH(OSi)); MS (CI, NH$_3$) *m/z* (relative intensity) 162 (MNH$_4^+$, 100), 145 (MH$^+$, 31); HRMS (EI) *m/z* (relative intensity) 127.0763 (5), 113.0604 (9), 110.0526 (100).

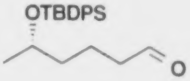


Methyl (*S*)-5-(*tert*-Butyldiphenylsilyloxy)hex-2-enoate (55) A modification of the procedure employed for **17** was adopted. A mixture of **45** (225 mg, 1.56 mmol), *t*-butyldiphenylsilyl chloride (0.82 mL, 2 equiv) and imidazole (213 mg, 3.12 mmol) in DMF (20 mL) was stirred at 50 °C for 20 h. Hexane (50 mL) was added and the mixture was then washed three times with H_2O, and dried (Na_2SO_4). Purification by column chromatography (10% Et_2O in hexane) gave **55** (501 mg, 84%). IR ($CHCl_3$ cast) 2931 (m), 1726 (s), 1659 (w), 1111 (s), 702 (s) cm^{-1}; ^{1}H NMR (300 MHz, CD_2Cl_2) δ 7.70 (m, 4 H, Ar-$\underline{H}$), 7.49-7.36 (m, 6 H, Ar-$\underline{H}$), 6.93 (dt, 1 H, J = 15.7, 7.7 Hz, $C\underline{H}$=CHCO), 5.79 (dt, 1 H, J = 15.7, 1.4 Hz, CH=$C\underline{H}$CO), 4.01 (m, 1 H, $C\underline{H}$(OSi)), 3.70 (s, 3 H, $OC\underline{H}_3$), 2.35 (m, 2 H, $C\underline{H}_2$CH), 1.11 (d, 3 H, J = 6.2 Hz, $C\underline{H}_3$CH(OSi)), 1.07 (s, 9 H, $(C\underline{H}_3)_3$CSi); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 166.90 ($\underline{C}$O), 146.03 ($\underline{C}$H=CHCO), 136.27 (*ortho*-$\underline{C}$), 134.48 (*ipso*-$\underline{C}$), 130.05 (*para*-$\underline{C}$), 128.07 (*meta*-$\underline{C}$), 123.47 (CH=$\underline{C}$HCO), 69.03 ($\underline{C}$H(OSi)), 51.56 ($O\underline{C}H_3$), 42.46 (CH_2CH), 27.17 ($(\underline{C}H_3)_3$CSi), 23.39 ($\underline{C}H_3$CH(OSi)), 19.46 ($(CH_3)_3\underline{C}$Si); MS (CI, NH_3) m/z (relative intensity) 400 (MNH_4^+, 100), 383 (MH^+, 31); HRMS (EI) m/z (relative intensity) 351.1783 (1), 325.1258 (87), 213.0737 (100).

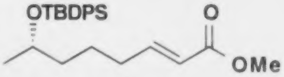


Methyl (*S*)-5-(*tert*-Butyldiphenylsilyloxy)hexanoate (56) The procedure employed for the conversion of **20a** to **21b** was used. Thus, olefin **55** (496 mg, 1.30 mmol) was hydrogenated in the presence of 10% Pd/C (50 mg) to afford **56** (488 mg, 98%). IR ($CHCl_3$ cast) 2931 (m), 1741 (s), 1111 (s), 703 (s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (m, 4 H, Ar-$\underline{H}$), 7.48-7.34 (m, 6 H, Ar-$\underline{H}$), 3.87 (m, 1 H, $C\underline{H}$(OSi)), 3.66 (s, 3 H, $OC\underline{H}_3$), 2.22 (t, 2 H, J = 7.4 Hz, $C\underline{H}_2$CO), 1.65 (m, 2 H, CH(OSi)$C\underline{H}_2$), 1.58-1.40 (m, 2 H, $C\underline{H}_2CH_2$CO), 1.08 (d, 3 H, J = 5.6 Hz, $C\underline{H}_3$CH(OSi)), 1.07 (s, 9 H, $(C\underline{H}_3)_3$CSi); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.03 ($\underline{C}$O), 135.84 (*ortho*-$\underline{C}$), 134.71 (*ipso*-$\underline{C}$), 134.40 (*ipso*-$\underline{C}$'), 129.49 (*para*-$\underline{C}$), 129.40 (*para*-$\underline{C}$'), 127.49 (*meta*-$\underline{C}$), 127.39 (*meta*-$\underline{C}$'), 69.06

($\underline{C}$H(OSi)), 51.38 (O$\underline{C}$H$_3$), 38.71 (CH(OSi)$\underline{C}$H$_2$), 34.03 ($\underline{C}$H$_2$CO), 27.01 (($\underline{C}$H$_3$)$_3$CSi), 23.07 ($\underline{C}$H$_3$CH(OSi)), 20.62 ($\underline{C}$H$_2$CH$_2$CO), 19.23 (CH$_3$)$_3\underline{C}$Si); MS (CI, NH$_3$) *m/z* (relative intensity) 402 (MNH$_4^+$, 10), 385 (MH$^+$, 50); HRMS (EI) *m/z* (relative intensity) 353.1932 (7), 327.1421 (100).

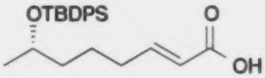


Methyl (*S*)-5-(*tert*-Butyldiphenylsilyloxy)hexanal (57) The procedures employed for the conversion of **17** to **19** were used. Thus, **56** (460 mg, 1.20 mmol) was reduced with DIBAL to the alcohol, then oxidized under Swern conditions to gave aldehyde **57** (346 mg, 82%). IR (CHCl$_3$ cast) 2931 (m), 2858 (m), 1726 (m), 1428 (m), 1111 (s), 702 (s) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 9.68 (t, 1 H, *J* = 1.8 Hz, C$\underline{H}$O), 7.70 (m, 4 H, Ar-$\underline{H}$), 7.48-7.34 (m, 6 H, Ar-$\underline{H}$), 3.87 (m, 1 H, C$\underline{H}$(OSi)), 2.30 (dt, 2 H, *J* = 7.3, 1.8 Hz, C$\underline{H}_2$CHO), 1.70-1.35 (m, 4 H, C$\underline{H}_2$C$\underline{H}_2$CH$_2$CO), 1.09 (d, 3 H, *J* = 6.1 Hz, C$\underline{H}_3$CH(OSi)), 1.07 (s, 9 H, (C$\underline{H}_3$)$_3$CSi); ^{13}C NMR (75 MHz, CDCl$_3$) δ 202.60 ($\underline{C}$HO), 135.86 (*ortho*-$\underline{C}$), 134.68 (*ipso*-$\underline{C}$), 134.35 (*ipso*-$\underline{C}$'), 129.55 (*para*-$\underline{C}$), 129.46 (*para*-$\underline{C}$'), 127.53 (*meta*-$\underline{C}$), 127.42 (*meta*-$\underline{C}$'), 69.00 ($\underline{C}$H(OSi)), 43.73 (CH(OSi)$\underline{C}$H$_2$), 38.67 ($\underline{C}$H$_2$CHO), 27.02 ($\underline{C}$H$_3$)$_3$CSi), 23.10 ($\underline{C}$H$_3$CH(OSi)), 19.24 (CH$_3$)$_3\underline{C}$Si), 17.77 ($\underline{C}$H$_2$CH$_2$CHO); MS (EI) *m/z* (relative intensity) 297.1312 (60).

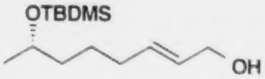


Methyl (*S*)-7-(*tert*-Butyldiphenylsilyloxy)oct-2-enoate (58) The procedure employed for the conversion of **19** to **20** was used. Thus, **57** (144 mg, 0.407 mmol) was coupled with (carbomethoxymethylene)triphenylphosphorane (150 mg, 0.45 mmol) to give **58** as a mixture of *E*-isomer (94.4 mg, 57%) and *Z*-isomer (45.0 mg, 27%). For the *E*-isomer: IR (CHCl$_3$ cast) 2932 (m), 2858 (m), 1726 (s), 1658 (w), 1111 (s), 702 (s) cm^{-1}; ^{1}H NMR

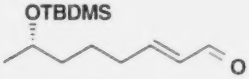
(300 MHz, CDCl$_3$) δ 7.68 (m, 4 H, Ar-H), 7.48-7.34 (m, 6 H, Ar-H), 6.92 (dt, 1 H, *J* = 15.7, 6.9 Hz, CH=CHCO), 5.77 (dt, 1 H, *J* = 15.7, 1.5 Hz, CH=CHCO), 3.85 (m, 1 H, CH(OSi)), 3.74 (s, 3 H, OCH$_3$), 2.10 (m, 2 H, CH$_2$CH), 1.55-1.38 (m, 4 H, CH(OSi)CH$_2$CH$_2$), 1.07 (d, 3 H, *J* = 5.5 Hz, CH$_3$CH(OSi)), 1.06 (s, 9 H, (CH$_3$)$_3$CSi); ^{13}C NMR (75 MHz, CDCl$_3$) δ 167.12 (CO), 149.49 (CH=CHCO), 135.87 (*ortho*-C), 135.84 (*ortho*-C'), 134.77 (*ipso*-C), 134.35 (*ipso*-C'), 129.51 (*para*-C), 129.43 (*para*-C'), 127.51 (*meta*-C), 127.40 (*meta*-C'), 120.90 (CH=CHCO), 69.15 (CH(OSi)), 51.35 (OCH$_3$), 38.77 (CH(OSi)CH$_2$), 32.13 (CH$_2$CH), 27.03 (CH$_3$)$_3$CSi), 23.59 (CH$_2$CH$_2$CH), 23.22 (CH$_3$CH(OSi)), 19.46 ((CH$_3$)$_3$CSi); MS (CI, NH$_3$) *m/z* (relative intensity) 428 (MNH$_4^+$, 20), 411 (MH$^+$, 2); HRMS (EI) *m/z* (relative intensity) 379.2095 (2), 353.1569 (100).



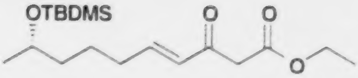
(*S*)-*E*-7-(*tert*-Butyldiphenylsilyloxy)oct-2-enoic Acid (59) The procedure employed for the conversion of **21b** to **22a** was used. Thus, **58** (*E*-isomer only, 90 mg, 0.22 mmol) was hydrolyzed to afford **59** (80 mg, 92%). ^{1}H NMR (360 MHz, CDCl$_3$) δ 7.68 (m, 4 H, Ar-H), 7.47-7.34 (m, 6 H, Ar-H), 7.02 (dt, 1 H, *J* = 15.6, 6.9 Hz, CH=CHCO), 5.77 (dt, 1 H, *J* = 15.6, 1.4 Hz, CH=CHCO), 3.86 (m, 1 H, CH(OSi)), 2.12 (m, 2 H, CH$_2$CH), 1.55-1.30 (m, 4 H, CH(OSi)CH$_2$CH$_2$), 1.08 (d, 3 H, *J* = 5.1 Hz, CH$_3$CH(OSi)), 1.06 (s, 9 H, (CH$_3$)$_3$CSi); ^{13}C NMR (75 MHz, CDCl$_3$) δ 171.20 (CO), 152.12 (CH=CHCO), 135.87 (*ortho*-C), 134.73 (*ipso*-C), 134.40 (*ipso*-C'), 129.54 (*para*-C), 129.44 (*para*-C'), 127.51 (*meta*-C), 127.40 (*meta*-C'), 120.47 (CH=CHCO), 69.10 (CH(OSi)), 38.75 (CH(OSi)CH$_2$), 32.22 (CH$_2$CH), 27.03 ((CH$_3$)$_3$CSi), 23.45 (CH$_2$CH$_2$CH=CH), 23.21 (CH$_3$CH(OSi)), 19.24 ((CH$_3$)$_3$CSi).



(*S*)-*E*-7-(*tert*-Butyldimethylsilyloxy)oct-2-enol (**62**) The procedure employed for the conversion of **17** to **18** was used. Thus, unlabeled unsaturated ester **32a** (*E*-isomer only, 30 mg, 0.11 mmol) was reduced with DIBAL (0.10 mL, 0.56 mmol) to give **62** (29 mg, crude), which was used directly for the next step. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.78-5.48 (m, 2 H, $C\underline{H}=C\underline{H}CH_2OH$), 4.10 (d, 2 H, *J* = 4.7 Hz, $C\underline{H}_2OH$), 3.78 (m, 1 H, $C\underline{H}(OSi)$), 2.05 (m, 2 H, $C\underline{H}_2CH=CH$), 1.65-1.20 (m, 5 H, $CH(OSi)C\underline{H}_2C\underline{H}_2 + O\underline{H}$), 1.13 (d, 3 H, *J* = 6.1 Hz, $C\underline{H}_3CH(OSi)$), 0.90 (s, 9 H, $(C\underline{H}_3)_3CSi$), 0.05 (s, 6 H, $Si(C\underline{H}_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 133.35 ($\underline{C}H=CHCH_2OH$), 129.02 ($CH=\underline{C}HCH_2OH$), 68.45 ($\underline{C}H(OSi)$), 63.86 (CH_2OH), 39.19 ($CH(OSi)\underline{C}H_2$), 32.23 ($\underline{C}H_2CH=CH$), 25.92 ($(\underline{C}H_3)_3CSi$), 23.83 ($\underline{C}H_2CH_2CH=CH$), 23.83 ($\underline{C}H_3CH(OSi)$), 18.40 ($(CH_3)_3\underline{C}Si$), -4.37 and -4.70 ($Si(\underline{C}H_3)_2$).

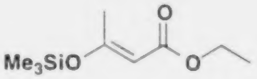


(*S*)-*E*-7-(*tert*-Butyldimethylsilyloxy)oct-2-enal (**63**) The procedure employed for the conversion of **18** to **19** was used. Thus, **62** (29 mg, crude) was oxidized under Swern conditions to give **63** (15.7 mg, 59% from **58**). IR ($CHCl_3$ cast) 2955 (m), 2929 (s), 2857 (m), 1694 (s), 835 (s), 774 (m) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.52 (d, 1 H, *J* = 7.9 Hz, $C\underline{H}O$), 6.85 (dt, 1 H, *J* = 15.6, 6.7 Hz, $C\underline{H}=CHCHO$), 6.13 (ddt, 1 H, *J* = 15.6, 7.9, 1.5 Hz, $CH=C\underline{H}CHO$), 3.81 (m, 1 H, $C\underline{H}(OSi)$), 2.34 (m, 2 H, $C\underline{H}_2CH=CH$), 1.80-1.30 (m, 4 H, $CH(OSi)C\underline{H}_2C\underline{H}_2$), 1.13 (d, 3 H, *J* = 6.1 Hz, $C\underline{H}_3CH(OSi)$), 0.89 (s, 9 H, $(C\underline{H}_3)_3CSi$), 0.05 (s, 6 H, $Si(C\underline{H}_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.06 ($\underline{C}HO$), 158.67 ($\underline{C}H=CHCHO$), 133.05 ($CH=\underline{C}HCHO$), 68.14 ($\underline{C}H(OSi)$), 39.01 ($CH(OSi)\underline{C}H_2$), 32.73 ($\underline{C}H_2CH=CH$), 25.86 ($(\underline{C}H_3)_3CSi$), 23.98 ($\underline{C}H_2CH_2CH=CH$), 23.80 ($\underline{C}H_3CH(OSi)$), 18.10 ($(CH_3)_3\underline{C}Si$), -4.36 and -4.74 ($(Si(\underline{C}H_3)_2$).

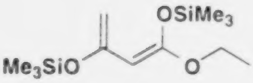


Ethyl (*S*)-9-(*tert*-Butyldimethysilyloxy)-3-oxodec-4-enoate (64) To a suspension of NaH (60% dispersion in mineral oil, 33 mg, 0.79 mmol) in THF (5 mL) was added a solution of **73** (105 mg, 0.395 mmol). After 30 min stirring, the aldehyde **31a** (91 mg, 0.40 mmol) in THF (5 mL) was added. The mixture was then stirred at room temperature overnight. The reaction mixture was acidified to pH 2 with 2N HCl, then extracted with CH_2Cl_2 (3 x 20 mL). The combined organic extracts were washed with brine, and dried (Na_2SO_4). Purification by Si_2O column chromatography (5% Et_2O in hexane) gave **64** (68 mg, 50%). IR ($CHCl_3$ cast) 2930 (m), 2857 (m), 1744 (m), 1666 (m), 1599 (m), 1236 (s), 836 (s) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) keto:enol = 30:70; for keto form δ 6.88 (dt, 1 H, J = 15.9, 6.9 Hz, $C\underline{H}{=}CHCO$), 6.16 (dt, 1 H, J = 15.9, 1.5 Hz, $CH{=}C\underline{H}CO$), 4.20 (q, 2 H, J = 7.1 Hz, $OC\underline{H}_2CH_3$), 3.79 (m, 1 H, $C\underline{H}(OSi)$), 3.58 (s, 2 H, $COC\underline{H}_2CO$), 2.22 (m, 2 H, $C\underline{H}_2CH{=}CH$), 1.70-1.40 (m, 4 H, $CH(OSi)C\underline{H}_2C\underline{H}_2$), 1.27 (t, 3 H, J = 7.1 Hz, $OCH_2C\underline{H}_3$), 1.12 (d, 3 H, J = 6.1 Hz, $C\underline{H}_3CH(OSi)$), 0.89 (s, 9 H, $(C\underline{H}_3)_3C$), 0.05 (s, 6 H, $(CH_3)_2Si$); for enol form δ 11.91 (d, 1 H J = 1.5 Hz, $O\underline{H}$), 6.65 (dt, 1 H, J = 15.5, 7.1 Hz, $C\underline{H}{=}CHC(OH)$), 5.75 (dd, 1 H, J = 15.5, 1.5 Hz, $CH{=}C\underline{H}C(OH)$), 4.98 (s, 1 H, $C(OH){=}C\underline{H}$), 4.21 (q, 2 H, J = 7.1 Hz, $OC\underline{H}_2CH_3$), 3.79 (m, 1 H, $C\underline{H}(OSi)$), 2.19 (m, 2 H, $C\underline{H}_2CH{=}CH$), 1.70-1.40 (m, 4 H, $CH(OSi)C\underline{H}_2C\underline{H}_2$), 1.30 (t, 3 H, J = 7.1 Hz, $OCH_2C\underline{H}_3$), 1.12 (d, 3 H, J = 6.1 Hz, $C\underline{H}_3CH(OSi)$), 0.89 (s, 9 H, $(C\underline{H}_3)_3C$), 0.05 (s, 6 H, $(CH_3)_2Si$); ^{13}C NMR (75 MHz, $CDCl_3$) for keto form δ 192.11 ($CH{=}CH\underline{C}OCH_2$), 167.40 ($\underline{C}OOCH_2$), 149.80 ($\underline{C}H{=}CHCO$), 129.64 ($CH{=}\underline{C}HCO$), 68.16 ($\underline{C}H(OSi)$), 61.29 ($O\underline{C}H_2CH_3$), 46.97 ($CO\underline{C}H_2CO$), 39.05 ($CH(OSi)\underline{C}H_2$), 32.59 ($\underline{C}H_2CH{=}CH$), 25.87 ($(\underline{C}H_3)_3C$), 24.10 ($CH(OSi)CH_2\underline{C}H_2$), 23.80 ($\underline{C}H_3CH(OSi)$), 18.10 ($(CH_3)_3\underline{C}$), 14.08 ($OCH_2\underline{C}H_3$), -4.39 and -4.74 ($(\underline{C}H_3)_2Si$); for enol form δ 173.01 ($\underline{C}(OH){=}CH$), 169.53 ($\underline{C}OOCH_2$), 140.91 ($\underline{C}H{=}CHC(OH)$), 124.42 ($CH{=}\underline{C}HC(OH)$), 90.00 ($C(OH){=}\underline{C}H$), 68.30 ($\underline{C}H(OSi)$), 60.00 ($O\underline{C}H_2CH_3$), 39.13 ($CH(OSi)\underline{C}H_2$), 32.59 ($\underline{C}H_2CH{=}CH$), 25.87 ($(\underline{C}H_3)_3C$), 24.65

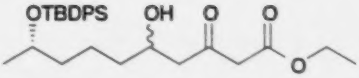
($CH(OSi)CH_2\underline{C}H_2$), 23.80 ($\underline{C}H_3CH(OSi)$), 18.10 ($(CH_3)_3\underline{C}$), 14.27 ($OCH_2\underline{C}H_3$), -4.39 and -4.74 ($(\underline{C}H_3)_2Si$); MS (CI, NH_3) *m/z* (relative intensity) 360 (MNH_4^+, 10), 343 (MH^+, 3); HRMS (EI) *m/z* (relative intensity) calcd for $C_{18}H_{34}O_4Si$ 342.2226, found 342.2224 (M^+, 0.8), 327.1988 (3), 285.1526 (100).



Ethyl 3-Trimethylsilyloxybut-2-enoate (**65**) The procedure of Danishefsky *et al.*[54b] was adopted. Freshly fused zinc chloride (0.50 g) was stirred with dry triethylamine (30.6 mL, 220 mmol) for 1h. To the cloudy suspension was added a solution of ethyl acetoacetate (13.0 g, 100 mmol) in benzene (35 mL), followed by trimethylsilyl chloride (25.6 mL, 200 mmol). The mixture was then stirred at 40 °C overnight. The mixture was cooled to 20 °C, ether (500 mL) was added. The precipitate formed was removed by filtration and washed twice with ether (100 mL). The filtrate and combined washings were concentrated *in vacuo*, and the residue was distilled through a 5 cm long Vigreux column to give **65** (17.03 g, 84%) as a mixture of two isomers (E:Z = 79:21), Bp 90-93 °C at 8 mm Hg. For *E*-isomer: ^1H NMR (300 MHz, $CDCl_3$) δ 5.09 (s, 1 H, $C(OSi){=}C\underline{H}$), 4.09 (q, 2 H, J = 7.1 Hz, $OC\underline{H}_2CH_3$), 2.23 (s, 3 H, $C\underline{H}_3C(OSi){=}CH$), 1.23 (t, 3 H, J = 7.1 Hz, $OCH_2C\underline{H}_3$), 0.23 (s, 9 H, $(C\underline{H}_3)_3Si$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.40 ($\underline{C}O$), 167.78 ($\underline{C}(OSi){=}CH$), 99.66 ($C(OSi){=}\underline{C}H$), 59.12 ($O\underline{C}H_2CH_3$), 20.54 ($\underline{C}H_3C(OSi)$), 14.29 ($OCH_2\underline{C}H_3$), 0.06 ($(\underline{C}H_3)_3Si$). For Z-isomer: ^1H NMR (300 MHz, $CDCl_3$) δ 5.05 (s, 1 H, $C(OSi){=}C\underline{H}$), 4.08 (q, 2 H, J = 7.1 Hz, $OC\underline{H}_2CH_3$), 1.86 (s, 3 H, $C\underline{H}_3C(OSi){=}CH$), 1.22 (t, 3 H, J = 7.1 Hz, $OCH_2C\underline{H}_3$), 0.24 (s, 9 H, $(C\underline{H}_3)_3Si$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.37 ($\underline{C}O$), 164.35 ($\underline{C}(OSi){=}CH$), 100.27 ($C(OSi){=}\underline{C}H$), 58.87 ($O\underline{C}H_2CH_3$), 24.23 ($\underline{C}H_3C(OSi)$), 14.29 ($OCH_2\underline{C}H_3$), 0.45 ($(\underline{C}H_3)_3Si$).

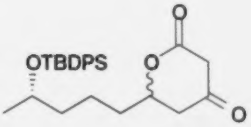


1-Ethoxy-1,3-bis(trimethylsilyloxy)-1,3-butadiene (66) The procedure of Chan *et al.*[54b] was followed. To a solution of diisopropylamine (4.20 mL, 29.6 mmol) in dry THF (100 mL) at 0°C, was added 2.5 M BuLi-hexane solution (12.0 mL, 29.6 mmol). The mixture was then cooled to -78 °C, **65** (5.00 g, 24.8 mmol) was added and the solution stirred for 2 min. The reaction was then quenched with trimethylsilyl chloride (5.0 mL, 39 mmol). After stirring for 30 min, the solvent was removed *in vacuo*. The residue was washed and filtered with cold dry hexane (0 °C). Concentration of the filtrate gave **66** (7.17 g, quantitative) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.47 (s, 1 H, $C(OSi)=C\underline{H}$), 4.13 (d, 1 H, J = 1.4 Hz, $C\underline{H}H=C(OSi)$), 3.91 (d, 1 H, J = 1.4 Hz, $CH\underline{H}=C(OSi)$), 3.77 (q, 2 H, J = 7.0 Hz, $OC\underline{H}_2CH_3$), 1.30 (t, 3 H, J = 7.0 Hz, $OCH_2C\underline{H}_3$), 0.26 (s, 9 H, $(C\underline{H}_3)_3Si$), 0.21 (s, 9 H, $(C\underline{H}_3)_3Si$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.51 ($\underline{C}(OSi)OCH_2CH_3$), 153.44 ($CH_2=\underline{C}(OSi)$), 89.08 ($\underline{C}H=C(OSi)O$), 77.90 ($\underline{C}H_2=C(OSi)$), 63.42 ($O\underline{C}H_2CH_3$), 14.27 ($OCH_2\underline{C}H_3$), 0.50 ($(\underline{C}H_3)_3Si$), 0.20 ($(\underline{C}H_3)_3Si$).



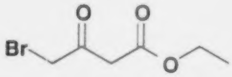
Ethyl (9*S*)-9-(*tert*-Butyldiphenylsilyloxy)-5-hydroxy-3-oxodecanoate (67) The procedure of Brownbridge *et al.*[53] was followed. To a cold (-78 °C) solution of aldehyde **57** (35.4 mg, 0.10 mmol) and disilyl ether **66** (30 mg, 0.10 mmol) in CH_2Cl_2 (5 mL) was added titanium tetrachloride (15 μL, 0.14 mmol). The orange solution was stirred at -78 °C for 2 h, and then aqueous Na_2CO_3 was added to quench the reaction. The mixture was extracted with ether (2 x 20 mL), and the combined extracts were dried (Na_2SO_4). After concentration, the residue was purified by column chromatography (3:1 hexane-Et_2O) to give **67** (17.2 mg, 36%) as a mixture of two diastereomers (ca. 1:1), existing exclusively in the keto form. IR ($CHCl_3$ cast) 3400 (br w), 2932 (s), 2858 (m), 1743 (s), 1713 (m),

1111 (s), 703 (s) $\mathrm{cm^{-1}}$; ^{1}H NMR (300 MHz, $\mathrm{CDCl_3}$) δ 7.68 (m, 4 H, Ar-$\underline{\mathrm{H}}$), 7.47-7.33 (m, 6 H, Ar-$\underline{\mathrm{H}}$), 4.21 (q, 2 H, *J* = 7.1 Hz, $\mathrm{OC\underline{H}_2CH_3}$), 3.97 (m, 1 H, $\mathrm{C\underline{H}(OSi)}$), 3.84 (m, 1 H, CH(OH)), 3.46 (s, 2 H, $\mathrm{COC\underline{H}_2CO}$), 2.70-2.51 (m, 2 H, $\mathrm{CH(OH)C\underline{H}_2CO}$), 1.57-1.20 (m, 7 H, $\mathrm{CH(OSi)C\underline{H}_2C\underline{H}_2C\underline{H}_2 + O\underline{H}}$), 1.29 (t, 3 H, *J* = 7.1 Hz, $\mathrm{OCH_2C\underline{H}_3}$), 1.08 (d, 3 H, *J* = 6.2 Hz, $\mathrm{C\underline{H}_3CH(OSi)}$), 1.06 (s, 9 H, $\mathrm{(C\underline{H}_3)_3C}$); ^{13}C NMR (75 MHz, $\mathrm{CDCl_3}$) δ 203.66 ($\mathrm{CH_2\underline{C}OCH_2}$), 166.88 ($\mathrm{\underline{C}OOCH_2}$), 135.87 (*ortho*-$\underline{\mathrm{C}}$)), 134.59 (*ipso*-$\underline{\mathrm{C}}$), 129.46 and 129.40 (*para*-$\underline{\mathrm{C}}$), 127.47 and 127.40 (*meta*-$\underline{\mathrm{C}}$), 69.43 and 69.32 ($\mathrm{\underline{C}H(OSi)}$), 67.37 ($\mathrm{\underline{C}H(OH)}$), 61.50 ($\mathrm{O\underline{C}H_2CH_3}$), 49.91 ($\mathrm{CO\underline{C}H_2CO}$), 49.52 ($\mathrm{CH(OH)\underline{C}H_2CO}$), 39.17 and 39.12 ($\mathrm{CH(OSi)\underline{C}H_2}$), 36.40 and 36.32 ($\mathrm{\underline{C}H_2C(OH)}$), 27.02 ($\mathrm{(\underline{C}H_3)_3C}$), 23.20 ($\mathrm{\underline{C}H_3CH(OSi)}$), 21.09 and 21.03 ($\mathrm{CH(OSi)CH_2\underline{C}H_2}$), 19.25 ($\mathrm{(CH_3)_3\underline{C}}$), 14.08 ($\mathrm{OCH_2\underline{C}H_3}$); MS (CI, $\mathrm{NH_3}$) *m/z* (relative intensity) 502 ($\mathrm{MNH_4^+}$, 32), 485 ($\mathrm{MH^+}$, 5); HRMS (EI) *m/z* (relative intensity) 409.1828 (9), 311.1110 (22), 297.1311 (38), 199.0580 (100).

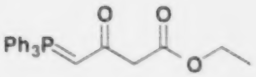


6-(4-(*tert*-Butyldimethylsilyloxyl)pentyl)-tetrahydro-2*H*-pyran-2,4-dione (68)

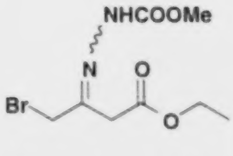
Compound **67** cyclized readily in $\mathrm{CDCl_3}$. In the NMR tube >95% cyclized in one week. IR ($\mathrm{CHCl_3}$ cast) 2931 (s), 2857 (m), 1666 (m), 1618 (m), 1111 (s), 703 (s) $\mathrm{cm^{-1}}$; ^{1}H NMR (360 MHz, $\mathrm{CDCl_3}$) δ 7.68 (m, 4 H, Ar-$\underline{\mathrm{H}}$), 7.48-7.34 (m, 6 H, Ar-$\underline{\mathrm{H}}$), 4.45 (m, 1 H, $\mathrm{C\underline{H}(OCO)}$), 3.88 (m, 1 H, $\mathrm{C\underline{H}(OSi)}$), 3.58-3.37 (m, 2 H, $\mathrm{COC\underline{H}_2CO}$), 2.65-2.30 (m, 2 H, $\mathrm{CH(OCO)C\underline{H}_2CO}$), 1.80-1.20 (m, 6 H, $\mathrm{CH(OSi)C\underline{H}_2C\underline{H}_2C\underline{H}_2}$), 1.12 (d, 3 H, *J* = 6.2 Hz, $\mathrm{C\underline{H}_3CH(OSi)}$), 1.06 (s, 9 H, $\mathrm{(C\underline{H}_3)_3C}$); ^{13}C NMR (75 MHz, $\mathrm{CDCl_3}$) δ 199.98 ($\mathrm{CH_2\underline{C}OCH_2}$), 167.11 ($\mathrm{\underline{C}OOCH}$), 135.85 (*ortho*-$\underline{\mathrm{C}}$)), 134.57 and 134.46 (*ipso*-$\underline{\mathrm{C}}$), 129.55 and 129.51 (*para*-$\underline{\mathrm{C}}$), 127.53 and 127.45 (*meta*-$\underline{\mathrm{C}}$), 75.28 ($\mathrm{\underline{C}H(OCO)}$), 69.07 and 68.87 ($\mathrm{\underline{C}H(OSi)}$), 46.98 ($\mathrm{CO\underline{C}H_2CO}$), 43.44 and 43.38 ($\mathrm{CH(OCO)\underline{C}H_2CO}$), 38.73 and 38.61 ($\mathrm{CH(OSi)\underline{C}H_2}$), 34.44 and 34.35 ($\mathrm{\underline{C}H_2C(OCO)}$), 27.01 ($\mathrm{(\underline{C}H_3)_3C}$), 23.19 ($\mathrm{\underline{C}H_3CH(OSi)}$), 20.33 and 20.29 ($\mathrm{CH(OSi)CH_2\underline{C}H_2}$), 19.23 ($\mathrm{(CH_3)_3\underline{C}}$); MS (CI, $\mathrm{NH_3}$) *m/z* (relative intensity) 456 (MNH_4^+, 70), 317 (100); HRMS (EI) *m/z* (relative intensity) 409.1836 (5), 381.1522 (39), 337.1625 (13), 199.0577 (100).



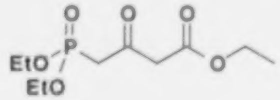
Ethyl 4-Bromo-3-oxobutanoate (70) The method of Momose *et al.*[56] was modified. To a cold (0 °C) solution of ethyl acetoacetate (6.51 g, 50.0 mmol) in CH_2Cl_2 (30 mL) was added a solution of bromine (2.58 mL, 50 mmol) in CH_2Cl_2 (10 mL) over a period of 30 min. The mixture was then kept at 4 °C overnight. After warming the mixture to room temperature, HBr was removed by bubbling of argon through the solution for 1h. After concentration, an orange oil (10.67 g) was obtained, which was purified by SiO_2 column chromatography (15% Et_2O in hexane) to afford **70** (8.42 g, 81%). IR ($CHCl_3$ cast) 2984 (m), 1746 (s), 1728 (s), 1324 (s), 1241 (s), 1028 (m) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) keto:enol = 80:20. For keto form δ 4.18 (q, 2 H, J = 7.2 Hz, $OC\underline{H}_2CH_3$), 4.04 (s, 2 H, $BrC\underline{H}_2CO$), 3.68 (s, 2 H, $COC\underline{H}_2CO$), 1.27 (t, 3 H, J = 7.2 Hz, $OCH_2C\underline{H}_3$); for enol form δ 11.97 (s, 1 H, $O\underline{H}$), 5.27 (s, 1 H, $C(OH)=C\underline{H}$), 4.21 (q, 2 H, J = 7.2 Hz, $OC\underline{H}_2CH_3$), 3.84 (s, 2 H, $BrC\underline{H}_2C(OH)$), 1.28 (t, 3 H, J = 7.2 Hz, $OCH_2C\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$) for keto form δ 194.53 ($BrCH_2\underline{C}O$), 166.50 ($\underline{C}OOCH_2$), 61.67 ($O\underline{C}H_2CH_3$), 45.98 ($CO\underline{C}H_2CO$), 33.86 ($Br\underline{C}H_2CO$), 13.96 ($OCH_2\underline{C}H_3$); for enol form δ 177.99 ($BrCH_2\underline{C}(OH)$), 170.40 ($\underline{C}OOCH_2$), 91.73 ($C(OH)=\underline{C}H$), 60.59 ($O\underline{C}H_2CH_3$), 28.30 ($Br\underline{C}H_2C(OH)$), 14.07 ($OCH_2\underline{C}H_3$); HRMS (EI) *m/z* (relative intensity) calcd for $C_6H_9{}^{81}BrO_3$ 209.9715, for $C_6H_9{}^{79}BrO_3$ 207.9735, found 209.9712 (M^+, 6), 207.9729 (M^+, 6), 129.0547 (36), 115.0391 (100). Anal. calcd for $C_6H_9BrO_3$: C, 34.47; H, 4.34; Br, 38.22. Found: C, 34.33; H, 4.33; Br, 38.38.



(3-Ethoxycarbonyl-2-oxopropylidene)triphenylphosphorane (71) The procedure of Sun *et al.*[55a] was modified. To a solution of triphenylphosphine (6.28 g, 23.9 mmol) in benzene (40 mL) was added **70** (5.00 g, 23.9 mmol) in benzene (10 mL) at such a rate that the temperature did not exceed 30 °C. The mixture was stirried at room temperature for 18 h and the resulting white precipitate was collected by filtration. It was washed twice with benzene, and dried to give a white solid (10.75 g, 94%). This Wittig salt was dissolved in a minimum amount of hot water (ca. 60 °C, 400 mL), a solution of Na$_2$CO$_3$ (3.62 g, 34.2 mmol) in water (20 mL) was then added, and the suspension was kept at 4 °C for 24 h before filtration. The collected precipitate was washed with water, and dried under vacuum to afford **71** (6.88 g, 77%) as a pale yellow powder. IR (KBr) 3376 (br w), 1711 (s), 1541 (s), 1437 (s), 1262 (m), 1108 (s) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.45-7.40 (m, 15 H, Ar$\underline{H}$), 4.19 (q, 2 H, J = 7.1 Hz, OC$\underline{H}_2$CH$_3$), 3.82 (br d, 1 H, J = 24.7 Hz, P=C$\underline{H}$), 3.36 (br s, 2 H, COC$\underline{H}_2$CO), 1.28 (t, 3 H, J = 7.1 Hz, OCH$_2$C$\underline{H}_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 183.90 (CH$\underline{C}$OCH$_2$), 170.64 ($\underline{C}$OOCH$_2$), 133.04 (d, J = 10.3 Hz, *meta*-$\underline{C}$), 132.10 (*para*-$\underline{C}$), 128.80 (d, J = 12.4 Hz, *ortho*-$\underline{C}$), 126.50 (d, J = 90.6 Hz, *ipso*-$\underline{C}$), 60.39 (O$\underline{C}$H$_2$CH$_3$), 52.38 (d, J = 107.8 Hz, P$\underline{C}$HCO), 48.26 (d, J = 15.3 Hz, CO$\underline{C}$H$_2$CO), 14.19 (OCH$_2\underline{C}$H$_3$); MS (EI) *m/z* (relative intensity) calcd for C$_{24}$H$_{23}$O$_3$P 390.1385, found 390.1379 (M$^+$, 20), 303.0932 (100). Anal. calcd for: C$_{24}$H$_{23}$O$_3$P: C, 73.83; H, 5.94. Found: C, 73.57; H, 5.90.

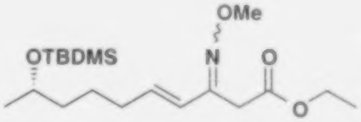


Ethyl 4-Bromo-3-oxobutanoate Methoxycarbonylhydrazone (72) The method of Corbel *et al.*[57] was followed. Compound **70** (2.09 g, 10.0 mmol) and methyl hydrazinocarboxylate (0.90 g, 10.0 mmol) were dissolved in ether (50 mL). After 5 h stirring at room temperature, the resulting precipitate was collected by filtration, washed with 1:1 ether-hexane (50 mL), and dried under vacuum over P_2O_5 in a desiccator to give **72** (2.42 g, 86%) as a white solid. A small portion was purified by recrystallization (hexane-ethyl acetate) for analysis. Mp 83-84 °C; IR ($CHCl_3$ cast) 3258 (br w), 1733 (s), 1530 (m), 1249 (s), 1177 (m) cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) *E*:*Z* = 82:18. For *E*-isomer δ 9.22 (br s, 1 H, N$\underline{H}$), 4.21 (q, 2 H, J = 7.1 Hz, OC$\underline{H}_2$CH$_3$), 4.16 (s, 2 H, BrC$\underline{H}_2$), 3.83 (s, 3 H, OC$\underline{H}_3$), 3.46 (s, 2 H, C$\underline{H}_2$CO), 1.29 (t, 3 H, J = 7.1 Hz, OCH$_2$C$\underline{H}_3$); For *Z*-isomer δ 9.22 (br s, 1 H, N$\underline{H}$), 4.17 (q, 2 H, J = 7.1 Hz, OC$\underline{H}_2$CH$_3$), 4.00 (s, 2 H, BrC$\underline{H}_2$), 3.85 (s, 3 H, OC$\underline{H}_3$), 3.50 (s, 2 H, C$\underline{H}_2$CO), 1.26 (t, 3 H, J = 7.1 Hz, OCH$_2$C$\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$) For *E*-isomer δ 166.64 ($\underline{C}$OOCH$_2$), 152.93 (NH$\underline{C}$OOCH$_3$), 141.28 ($\underline{C}$=N), 60.93 (O$\underline{C}$H$_2$CH$_3$), 51.68 (O$\underline{C}$H$_3$), 34.00 (Br$\underline{C}$H$_2$), 33.90 ($\underline{C}$H$_2$CO), 14.46 (OCH$_2$$\underline{C}H_3$); HRMS (EI) *m/z* (relative intensity) calcd for $C_8H_{13}{}^{79}BrN_2O_4$ 280.0059, found 280.0045 (M$^+$, 12), 201.0872 (100); Anal. Calcd for $C_8H_{13}BrN_2O_4$: C, 34.18; H, 4.66; Br, 28.42; N, 9.97. Found: C, 34.27; H, 4.30; Br, 28.73; N, 10.22.

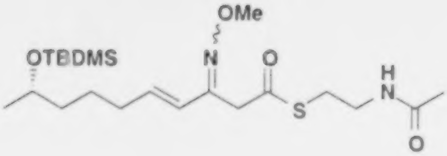


Ethyl 4-(Diethoxyphosphinyl)-3-oxobutanoate (**73**) The method of Corbel *et al.*[57] was used. To a refluxing solution of triethylphosphite (1.20 mL, 7.03 mmol) in toluene (50 mL) was added **72** (1.88 g, 6.69 mmol) in $CHCl_3$ (10 mL). The mixture was then heated at reflux for 3h. After removal of solvent, the residue was dissolved in acetone (50 mL), and 3 M HCl (30 mL) was then added. The mixture was stirred at room temperature for 3h. Acetone was evaporated *in vacuo*, and the residue was extracted with $CHCl_3$ (3 x 50 mL). The combined $CHCl_3$ layers were washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. Purification with SiO_2 column chromatography (2-5% CH_3OH in $CHCl_3$) gave **73** (1.46 g, 82%). IR ($CHCl_3$ cast) 2984 (m), 1744 (s), 1720 (s), 1257 (s),

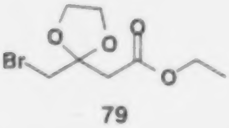
1026 (s) cm^{-1}; ^{1}H NMR (360 MHz, CDCl$_3$) δ 4.24-4.10 (m, 6 H, 3 x OC$\underline{H}_2$CH$_3$), 3.67 (s, 2 H, COC$\underline{H}_2$CO), 3.27 (d, 2 H, J = 22.7 Hz, P(O)C$\underline{H}_2$CO), 1.34 (t, 6 H, J = 7.0 Hz, 2 x P(O)OCH$_2$C$\underline{H}_3$), 1.28 (t, 3 H, J = 7.2 Hz, OCH$_2$C$\underline{H}_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ⊇194.52 (d, J = 6.0 Hz, CH$_2$$\underline{C}OCH_2$), 172.65 ($\underline{C}$OOCH$_2$), 62.67 (d, J = 6.3 Hz, P(O)O$\underline{C}$H$_2$CH$_3$), 61.37 (O$\underline{C}$H$_2$CH$_3$), 49.55 (CO$\underline{C}$H$_2$CO), 42.46 (d, J = 126.7 Hz, P(O)$\underline{C}$H$_2$CO), 16.12 (P(O)OCH$_2$$\underline{C}H_3$), 14.32 (OCH$_2$$\underline{C}H_3$); MS (EI) *m/z* (relative intensity) calcd for $C_{10}H_{19}O_6P$ 266.0919, found 266.0916 (M$^+$, 15), 221.0581 (63), 193.0264 (30), 179.0472 (100).



Ethyl (*S*)-9-(*tert*-Butyldimethylsilyloxy)-3-oxodec-4-enoate *O*-Methoxime (75) The procedure of Corey *et al.*[59] was followed. To a solution of **64** (19.4 mg, 0.0567 mmol) and methoxylamine hydrochloride (8.0 mg, 0.091 mmol) in methanol (1 mL) was added pyridine (10 μl, 0.12 mmol). The mixture was then stirred at room temperature for 3 h. After the addition of 0.25 M HCl (10 mL), the solution was extracted with CHCl$_3$ (3 x 10 mL). The combined extracts were dried (Na$_2$SO$_4$) and concentrated to give **75** (18.0 mg, 86%) as a colorless oil. ^{1}H NMR (360 MHz, CDCl$_3$): a mixture of *syn* and *anti* isomers (1:3). For the major isomer: δ 6.20-5.90 (m, 2 H, C$\underline{H}$=C$\underline{H}$), 4.17 (q, 2 H, J = 7.1 Hz, OC$\underline{H}_2$CH$_3$), 3.91 (s, 3 H, OC$\underline{H}_3$), 3.80 (m, 1 H, C$\underline{H}$(OSi)), 3.49 (s, 2 H, C$\underline{H}_2$CO), 2.18 (m, 2 H, C$\underline{H}_2$CH=CH), 1.80-1.30 (m, 4 H, CH(OSi)C$\underline{H}_2$C$\underline{H}_2$), 1.26 (t, 3 H, J = 7.1 Hz, OCH$_2$C$\underline{H}_3$), 1.12 (d, 3 H, J = 6.0 Hz, C$\underline{H}_3$CH(OSi)), 0.89 (s, 9 H, (C$\underline{H}_3$)$_3$C), 0.05 (s, 6 H, (CH$_3$)$_2$Si).



NAC (*S*)-9-(*tert*-Butyldimethylsilyloxy)-3-oxodec-4-enoate *O*-Methoxime (77) The procedure for the conversion for **32** to **43** was used. Thus, **75** (18.0 mg, 0.048 mmol) was converted to **77** (13 mg, 64% from **75**). IR ($CHCl_3$ cast) 3280 (br w), 2929 (s), 2855 (m), 1654 (s), 1554 (m), 1053 (s), cm^{-1}; ^{1}H NMR (360 MHz, CD_2Cl_2) δ 6.18-5.99 (m, 2 H, $C\underline{H}{=}C\underline{H}$), 5.77 (br s, 1 H, $N\underline{H}$), 3.88 (s, 3 H, $OC\underline{H}_3$), 3.80 (m, 1 H, $C\underline{H}(OSi)$), 3.76 (s, 2 H, $C\underline{H}_2CO$), 3.37 (q, 2 H, J = 6.3 Hz, $C\underline{H}_2NH$), 3.00 (t, 2 H, $SC\underline{H}_2$), 2.17 (m, 2 H, $C\underline{H}_2CH{=}CH$), 1.90 (s, 3 H, $COCH_3$), 1.80-1.23 (m, 4 H, $CH(OSi)C\underline{H}_2C\underline{H}_2$), 1.10 (d, 3 H, J = 6.0 Hz, $C\underline{H}_3CH(OSi)$), 0.88 (s, 9 H, $(C\underline{H}_3)_3C$), 0.05 (s, 6 H, $(CH_3)_2Si$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.35 ($\underline{C}OS$), 170.17 ($\underline{C}OCH_3$), 151.56 ($\underline{C}{=}N$), 137.75 ($\underline{C}H{=}CHC$), 126.46 ($CH{=}\underline{C}HC$), 68.68 ($\underline{C}H(OSi)$), 62.24 ($O\underline{C}H_3$), 40.06 ($\underline{C}H_2NH$), 39.67 ($CH(OSi)\underline{C}H_2$), 39.47 ($\underline{C}H_2CO$), 33.28 ($\underline{C}H_2CH{=}CH$), 29.14 ($S\underline{C}H_2$), 26.02 ($(\underline{C}H_3)_3C$), 25.31 ($CH(OSi)CH_2\underline{C}H_2$), 23.95 ($\underline{C}H_3CH(OSi)$), 23.27 ($CO\underline{C}H_3$), 18.33 ($(CH_3)_3\underline{C}$), -4.34 and -4.67 ($(\underline{C}H_3)_2Si$); MS (FAB, Cleland) *m/z* (relative intensity) 445 (MH^+, 53), 194 (100).



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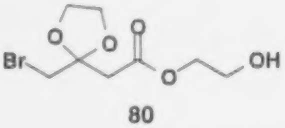
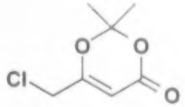


80

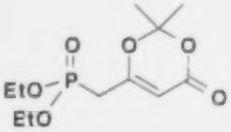
Ethyl 4-Bromo-3-oxobutanoate Ethylene Acetal (79) and 2-Hydroxyethyl 4-Bromo-3-oxobutanoate Ethylene Acetal (80) A solution of **70** (418 mg, 2.00 mmol) and *p* toluenesulfonic acid monohydrate (380 mg, 4.00 mmol) in benzene (10 mL) was heated to reflux in a Soxhlet apparatus containing CaH_2 for 5 h. After the addition of K_2CO_3 solution the mixture was extracted with $CHCl_3$, dried (Na_2SO_4) and concentrated *in vacuo*. Flash chromatography on SiO_2 (2% CH_3OH in $CHCl_3$) gave **79** (168 mg, 33%)

and **80** (180 mg, 33%). For **79**: IR ($\mathrm{CHCl_3}$ cast) 2980 (w), 1734 (s), 1198 (m), 1092 (m), 1034 (s) cm^{-1}; ^{1}H NMR (360 MHz, $\mathrm{CDCl_3}$) δ 4.16 (q, 2 H, *J* = 7.2 Hz, $\mathrm{OC\underline{H}_2CH_3}$), 4.12-4.04 (m, 4 H, $\mathrm{OC\underline{H}_2C\underline{H}_2O}$), 3.66 (s, 2 H, $\mathrm{BrC\underline{H}_2}$), 2.89 (s, 2 H, $\mathrm{C\underline{H}_2CO}$), 1.17 (t, 3 H, *J* = 7.2 Hz, $\mathrm{OCH_2C\underline{H}_3}$); ^{13}C NMR (75 MHz, $\mathrm{CDCl_3}$) δ 168.84 ($\mathrm{\underline{C}O}$), 106.84 ($\mathrm{BrCH_2\underline{C}}$), 65.92 ($\mathrm{O\underline{C}H_2\underline{C}H_2O}$) 60.78 ($\mathrm{O\underline{C}H_2CH_3}$), 41.08 ($\mathrm{\underline{C}H_2CO}$), 35.46 ($\mathrm{Br\underline{C}H_2}$), 14.12 ($\mathrm{OCH_2\underline{C}H_3}$); HRMS (EI) *m/z* (relative intensity) 238.9744 (13), 236.9746 (13), 166.9528 (67), 164.9546 (69), 159.0655 (100). For **80**: ^{1}H NMR (360 MHz, $\mathrm{CDCl_3}$) δ 4.24 (t, 2 H, *J* = 4.8 Hz, $\mathrm{OC\underline{H}_2CH_2OH}$), 4.11-4.03 (m, 4 H, $\mathrm{OC\underline{H}_2C\underline{H}_2O}$), 3.81 (t, 2 H, *J* = 4.8 Hz, $\mathrm{OCH_2C\underline{H}_2OH}$), 3.63 (s, 2 H, $\mathrm{BrC\underline{H}_2}$), 2.94 (s, 2 H, $\mathrm{C\underline{H}_2CO}$), 2.18 (br, 1 H, $\mathrm{O\underline{H}}$).

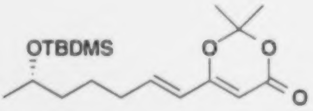


6-Chloromethyl-2,2-dimethyl-1,3-dioxen-4-one (**83**) The procedure of Boeckman *et al.*[61d] was followed. A solution of lithium diisopropylamide in THF (30 mL) was prepared *in situ* by mixing the corresponding amine (7.5 mL, 57 mmol) with *n*-BuLi (2.5 M in hexane, 22.8 mL, 57 mmol) at -75 °C. Diketene acetone adduct **82** (5.68 g, 38 mmol) in THF (10 mL) was then added. After 15 min stirring the resulting brown solution was cannulated into a solution of hexachloroethane (13.49 g, 57 mmol) in THF (50 mL) at -55 °C. The reaction mixture was then warmed to -25 °C over 30 min before it was poured into ice-cooled 10% HCl (80 mL). The aqueous layer was extracted with $\mathrm{Et_2O}$, the combined organic layers were washed once with saturated $\mathrm{NaHCO_3}$, dried ($\mathrm{Na_2SO_4}$) and concentrated *in vacuo*. Flash chromatography (10-20% EtOAc-hexane) gave **83** (4.90 g, 73%). IR ($\mathrm{CHCl_3}$ cast) 1731 (s), 1642 (m), 1393 (s), 1274 (s), 1203 (m), 1016 (s) cm^{-1}; ^{1}H NMR (300 MHz, $\mathrm{CDCl_3}$) δ 5.55 (t, 1 H, *J* = 0.5 Hz, $\mathrm{C{=}C\underline{H}}$), 4.02 (d, 2 H, *J* = 0.5 Hz, $\mathrm{C\underline{H}_2Cl}$), 1.71 (s, 6 H, $\mathrm{C(C\underline{H}_3)_2}$); ^{13}C NMR (75 MHz, $\mathrm{CDCl_3}$) δ 164.46 ($\mathrm{\underline{C}(OR){=}CH}$), 160.39 ($\mathrm{\underline{C}O}$), 107.94 ($\mathrm{O\underline{C}(CH_3)_2O}$), 94.76 ($\mathrm{C(OR){=}\underline{C}H}$), 40.96 ($\mathrm{\underline{C}H_2Cl}$), 24.79 (2 x $\mathrm{\underline{C}H_3}$);

MS (FAB, Cleland) *m/z* (relative intensity) 179 (MH^+ for ^{37}Cl, 21), 177 (MH^+ for ^{35}Cl, 65), HRMS (EI) *m/z* (relative intensity) calcd for $C_7H_8{}^{37}ClO_3$ 177.0132, $C_7H_8{}^{35}ClO_3$ 175.0162, found 177.0135 (M^+-H, 2), 175.0156 (M^+-H, 7), 127.0400 (43).

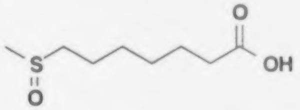


6-Diethylphophonomethyl-2,2-dimethyl-1,3-dioxen-4-one (84) The procedure of Boeckman *et al.*[61d] was followed. To a stirring mixture of potassium *tert*-butoxide (8.4 g, 71 mmol) in DMF (80 mL) cooled to 0 °C was added diethyl phosphite (10.1 g, 73.0 mmol). After 30 min a solution of **83** (4.18 g, 23.7 mmol) in THF (20 mL) was added. The mixture was stirred at 0 °C for further 30 min before it was filtered through a pad of celite, and dried (K_2CO_3). After concentration *in vacuo* (maintaining the temperature below 50 °C) the residue was purified by flash chromatography on florisil (60-100 mesh, 1:1 hexane-EtOAc to EtOAc) to afford **84** (5.51 g, 84%). IR (CH_2Cl_2 cast) 2987 (w), 1730 (s), 1634 (m), 1376 (s), 1270 (s), 1025 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.37 (d, 1 H, J = 3.6 Hz, C=C$\underline{H}$), 4.14 (dq, 4 H, J = 8.3, 7.1 Hz, 2 x $OC\underline{H}_2CH_3$), 2.78 (d, 2 H, J = 22.1 Hz, $P(O)C\underline{H}_2$), 1.68 (s, 6 H, $C(C\underline{H}_3)_2$), 1.33 (t, 6 H, J = 7.1 Hz, 2 x $OCH_2C\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.96 (d, J = 10.4 Hz, $\underline{C}(OR)=CH$), 160.52 ($\underline{C}O$), 107.09 ($O\underline{C}(CH_3)_2O$), 96.13 (d, J = 7.7 Hz, $C(OR)=\underline{C}H$), 62.61 (d, J = 6.3 Hz, 2 x $O\underline{C}H_2CH_3$), 32.38 (d, J = 136.7 Hz, $P(O)\underline{C}H_2$), 24.92 (2 x $\underline{C}H_3$), 16.32 (d, J = 6.5 Hz, 2 x OCH_2CH_3)



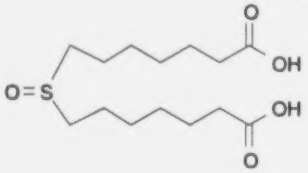
(*S*)-6-(6-*tert*-Butyldimethylsilyloxyl-1-hepten-1-yl)-2,2-dimethyl-1,3-dioxen-4-one (85) The procedure for the conversion of **73** to **64** was used. Thus, **84** (278 mg, 1.00

mmol) condensed with **31a** (115 mg, 0.50 mmol) to produce **85** (173 mg, 98%). IR ($CHCl_3$ cast) 2929 (m), 2857 (m), 1731 (s), 1654 (s), 1390 (s), 1374 (s), 836 (m) cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 6.55 (dt, 1 H, *J* = 15.5, 7.0 Hz, $CH_2C\underline{H}$=CH), 5.89 (dt, 1 H, *J* = 15.5, 1.4 Hz, CH_2CH=$C\underline{H}$), 5.24 (s, 1 H, C=$C\underline{H}$CO), 3.80 (m, 1 H, $C\underline{H}$(OSi)), 2.21 (m, 1H, $C\underline{H}_2CH$=CH), 1.71 (s, 6 H, $C(C\underline{H}_3)_2$), 1.30-1.60 (m, 4 H, $CH(OSi)C\underline{H}_2C\underline{H}_2$), 1.13 (d, 3 H, *J* = 6.0 Hz, $C\underline{H}_3CH(OSi)$), 0.89 (s, 9 H, $C(C\underline{H}_3)_3$), 0.05 (s, 3 H, $C\underline{H}_3SiCH_3$), 0.04 (s, 3 H, $CH_3SiC\underline{H}_3$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 163.40 (COO), 162.13 ($\underline{C}$(OR)=CH), 142.45 ($CH_2\underline{C}H$=CH), 122.55 (CH_2CH=$\underline{C}H$), 106.24 ($O\underline{C}(CH_3)_2O$), 93.25 (C(OR)=$\underline{C}$H), 68.22 ($\underline{C}H(OSi)$), 38.99 ($CH(OSi)\underline{C}H_2$), 32.69 ($\underline{C}H_2CH$=CH), 25.86 ($C(\underline{C}H_3)_3$), 24.98 (2 x $\underline{C}H_3$), 24.34 ($C\underline{H}_2CH_2CH$=CH), 23.07 (CH3CH(OSi)), 18.10 ($\underline{C}(CH_3)_3$), -4.39 and -4.77 ($Si(\underline{C}H_3)_2$).

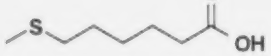


7-(Methylsulfinyl)heptanoic Acid (**95**) DMSO (200 mL) was carefully added to NaH (3.60 g, 0.30 mole), the suspension was warmed at 60 °C until no more gas was evolved, then cooled down to room temperature. A solution of 6-bromohexanoic acid (19.51 g, 0.10 mole) in DMSO (50 mL) was added over a period of 2 h, the mixture was stirred overnight. The reaction mixture was acidified to pH 1 with 2N HCl, and extracted with CH_2Cl_2 (4 x 200 mL), then dried (Na_2SO_4). After concentration the residue was further dried under high vacuum to give a dark orange oil (ca. 20 mL). The crude product was redissolved in 0.5 N NaOH solution (120 mL), and washed with CH_2Cl_2. The aqueous layer was then acidified to pH 1 and extracted with CH_2Cl_2. The dried extracts (Na_2SO_4) were then concentrated and purified with flash chromatography on SiO_2 (5-10% CH_3OH in EtOAc) to provide **95** (2.34 g, 12%). IR ($CHCl_3$, cast) 3200-2400 (br s), 2934 (s), 2861 (m), 1718 (s), 1005 (s) cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 11.31 (br, 1 H, $COO\underline{H}$), 2.90-2.60 (m, 2 H, $S(O)C\underline{H}_2$), 2.60 (s, 3 H, $C\underline{H}_3S(O)$), 2.30 (t, 2 H, *J* = 7.4 Hz, $C\underline{H}_2CO$), 1.74

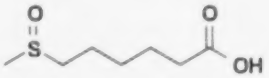
(m, 2 H, S(O)CH$_2$C$\underline{\text{H}}_2$), 1.62 (m, 2 H, C$\underline{\text{H}}_2$CH$_2$CO), 1.55-1.30 (m, 4 H, S(O)CH$_2$CH$_2$C$\underline{\text{H}}_2$C$\underline{\text{H}}_2$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 177.84 ($\underline{\text{C}}$OOH), 53.89 (S(O)$\underline{\text{C}}$H$_2$), 37.84 ($\underline{\text{C}}$H$_3$S(O)), 33.83 ($\underline{\text{C}}$H$_2$CO), 29.49, 28.28, 24.36, 22.31; MS (EI) *m/z* (relative intensity) calcd for $C_8H_{16}O_3S$ 192.0820, found 192.0814 (M$^+$, 5), 175.0791 (45).



Bis-(carboxyhex-6-yl) Sulfoxide (96) The above procedure was used except that the reaction was performed with three fold excess dimsyl sodium in THF at -10 °C. The reaction was found to be incomplete, and **96** was isolated in low (ca. 15%) yield. IR (CHCl$_3$, cast) 3400-2300 (br m), 2931 (s), 2860 (m), 1735 (s), 979 (s) cm^{-1}; ^{1}H NMR (360 MHz, CD$_3$OD) δ 2.86-2.70 (m, 4 H, 2 x S(O)C$\underline{\text{H}}_2$), 2.29 (t, 4 H, *J* = 7.4 Hz, 2 x C$\underline{\text{H}}_2$CO), 1.76 (m, 4 H, 2 x S(O)CH$_2$C$\underline{\text{H}}_2$), 1.62 (m, 4 H, 2 x C$\underline{\text{H}}_2$CH$_2$CO), 1.56-1.35 (m, 8 H, 2 x S(O)CH$_2$CH$_2$C$\underline{\text{H}}_2$C$\underline{\text{H}}_2$); ^{13}C NMR (75 MHz, CD$_3$OD) δ 177.37 ($\underline{\text{C}}$OOH), 52.63 (S(O)$\underline{\text{C}}$H$_2$), 34.73 ($\underline{\text{C}}$H$_2$CO), 29.68, 29.38, 25.74, 23.56; HRMS (EI) *m/z* (relative intensity) calcd for $C_{14}H_{26}O_5S$ 306.1501, found 306.1494 (M$^+$, 0.5), 289.1472 (44);. MS (FAB, Cleland) *m/z* (relative intensity) 307 (MH$^+$, 100). Anal. Calcd for $C_{14}H_{26}O_5S$, 54.88; H, 8.55; S, 10.46. Found: C, 54.94; H, 8.76; S, 10.08.



6-(Methylthio)hexanoic Acid (97) A modification of the procedure of Clements *et al.*[79] was used. A solution of 6-bromohexanoic acid (50.8 g, 0.261 mol) in methanol (300 mL) at 0 °C was carefully treated with liquid methanethiol (40 g, 0.83 mole), followed by 6N sodium hydroxide (98 mL). The mixture was then warmed to 20 °C and stirred overnight. Excess thiol was removed by bubbling argon into the solution (vent to bleach solution in hood), and methanol was evaporated *in vacuo*. The residue was acidified to pH 1 with HCl, and then extracted five times with hexane. The dried extracts ($\mathrm{Na_2SO_4}$) were concentrated to give **97** (40.2 g, 95%) of as a colorless oil: IR ($\mathrm{CHCl_3}$, cast) 3037 (br m), 2936 (m), 2918 (m), 1708 (s) cm^{-1}; ^{1}H NMR (360 MHz, $\mathrm{CD_2Cl_2}$) δ 11.85 (br, 1 H, $\mathrm{COO\underline{H}}$), 2.48 (t, 2 H, J = 7.3 Hz, $\mathrm{SC\underline{H}_2}$), 2.36 (t, 2 H, J = 7.4 Hz, $\mathrm{C\underline{H}_2CO}$), 2.06 (s, 3 H, $\mathrm{C\underline{H}_3S}$), 1.62 (m, 4 H, $\mathrm{SCH_2C\underline{H}_2 + C\underline{H}_2CH_2CO}$), 1.44 (m, 2 H, $\mathrm{SCH_2CH_2C\underline{H}_2}$); ^{13}C NMR (75 MHz, $\mathrm{CD_2Cl_2}$) δ 180.54 ($\mathrm{\underline{C}OOH}$), 34.29 ($\mathrm{S\underline{C}H_2}$), 34.27 ($\mathrm{\underline{C}H_2CO}$), 29.17 ($\mathrm{SCH_2\underline{C}H_2}$), 28.52 ($\mathrm{\underline{C}H_2CH_2CO}$), 24.65 ($\mathrm{SCH_2CH_2\underline{C}H_2}$), 15.53 ($\mathrm{\underline{C}H_3S}$); HRMS (EI) calcd for $\mathrm{C_7H_{14}O_2S}$ 162.0715, found 162.0714. Anal. Calcd for $\mathrm{C_7H_{14}O_2S}$: C, 51.82; H, 8.70; S, 19.76. Found: C, 51.66; H, 9.07; S, 19.51.

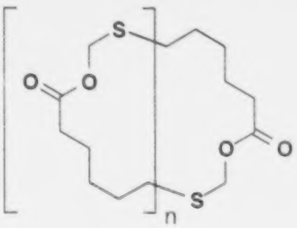


6-(Methylsulfinyl)hexanoic Acid (98) A solution of **97** (40.0 g, 0.247 mole) in methanol (500 mL) at 0 °C was treated with 0.50 M sodium metaperiodate (494 mL, 0.247 mole) overnight. It was concentrated to dryness below 10 °C, and the residue was extracted with $\mathrm{CH_2Cl_2}$ (4 x equal volume). Combined extracts were dried ($\mathrm{Na_2SO_4}$) and evaporated to give **98** (42.6 g, 97%) as an oil which solidified upon standing: mp 48-50 °C; IR ($\mathrm{CHCl_3}$ cast) 3200-2500 (br m), 2936 (s), 2865 (m), 1718 (s), 1002 (s) cm^{-1}; ^{1}H NMR (360 MHz, $\mathrm{CD_2Cl_2}$) δ 10.76 (br, 1 H, $\mathrm{COO\underline{H}}$), 2.88-2.65 (m, 2 H, $\mathrm{S(O)C\underline{H}_2}$), 2.59 (s, 3 H, $\mathrm{C\underline{H}_3S(O)}$), 2.28 (t, 2 H, J = 7.3 Hz, $\mathrm{C\underline{H}_2CO}$), 1.73 (m, 2 H, $\mathrm{S(O)CH_2C\underline{H}_2}$), 1.62 (m, 2 H, $\mathrm{C\underline{H}_2CH_2CO}$), 1.48 (m, 2 H, $\mathrm{S(O)CH_2CH_2C\underline{H}_2}$); ^{13}C NMR (75 MHz, $\mathrm{CD_2Cl_2}$) δ 176.44 ($\mathrm{\underline{C}OOH}$), 53.91 ($\mathrm{S(O)\underline{C}H_2}$), 38.01 ($\mathrm{\underline{C}H_3S(O)}$), 33.99 ($\mathrm{\underline{C}H_2CO}$), 28.41 ($\mathrm{S(O)CH_2\underline{C}H_2}$), 24.65 ($\mathrm{\underline{C}H_2CH_2CO}$), 22.53 ($\mathrm{S(O)CH_2CH_2\underline{C}H_2}$); HRMS (EI) calcd for $\mathrm{C_7H_{14}O_3S}$ 178.0664, found 178.0668; MS (FAB, Cleland) *m/z* (relative intensity) 179 (MH$^+$, 100). Anal. Calcd for $\mathrm{C_7H_{14}O_3S}$: C, 47.17; H, 7.92; S, 17.99. Found: C, 46.82; H, 8.00; S, 17.81.

General Procedure for the Modified Swern Oxidation A solution of **98** (1.07 g, 6.0 mmol) in dichloromethane (15 mL) was cooled to -50 to -60 °C, oxalyl chloride (5.5 mL, 1M solution in CH_2Cl_2, 5.5 mmol) was added dropwise, followed after 15 min by a solution of the alcohol (5 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred for 30 min, triethylamine (25 mmol) was added, and the solution was kept at -50 to -60 °C for 5 min before warming to room temperature. Workup involved filtration through a silica gel column with further elution with ethyl acetate (5 column volumes). Simple evaporation of the filtrate afforded the product, which was analyzed by ^{1}H NMR spectrometry using a long relaxation delay (12 s) to determine the alcohol/carbonyl product composition. A portion (200 mg) of the product was then further purified by flash chromatography (20 g of SiO_2, 30% Et_2O in hexane) to determine the isolated yields. All of the carbonyl products from the Swern oxidation were characterized by comparing chromatographic and/or spectral properties (^{1}H NMR, IR, MS) with the authentic samples or using published literature values.

The parent acid **97** was then recovered by washing the silica gel with 5 column volumes of 1% acetic acid in ethyl acetate then evaporating *in vacuo*. The residue was diluted with water and extracted five times with hexane. The extract was dried (Na_2SO_4) and concentrated to give **97**. The sulfide recovered this way was essentially pure, and the yield of recovery was normally >95%. For larger scale reactions, the carboxylic acid functionality in the reagent allows a convenient separation of products from **97** by acid-base extraction. Any excess sulfoxide reagent **98** is not recovered by this procedure since it is water soluble.

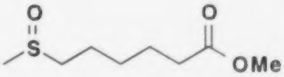
Improved Procedure for the Modified Swern Oxidation Same as general procedure except that after the addition of triethylamine the reaction mixture was kept at -40 °C for 2 h before warming to room temperature.



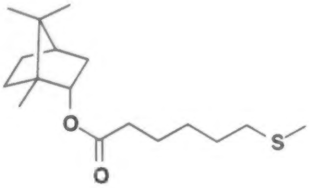
15 n = 1
16 n = 2

Pummerer Products 100 and 101 The general procedure for the modified Swern oxidation was followed except no alcohol was added. Dimer **100** and trimer **101** were isolated by SiO_2 flash chromatography (10-20% EtOAc in hexane). For **100** (13%): IR ($CHCl_3$ cast) 1739 (s), 1157 (m) cm^{-1}; ^{1}H NMR (360 MHz, CD_2Cl_2) δ 5.16 (s, 4 H, 2 x $OC\underline{H}_2S$), 2.68 (t, 4 H, *J* = 7.4 Hz, 2 x $C\underline{H}_2CO$), 2.33 (t, 4 H, *J* = 7.3 Hz, 2 x $SC\underline{H}_2$), 1.73-1.60 (m, 8 H, 2 x $C\underline{H}_2CH_2CO$ + 2 x $SCH_2C\underline{H}_2$), 1.52-1.38 (m, 4 H, 2 x $SCH_2CH_2C\underline{H}_2$); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 173.06 ($\underline{C}O$), 67.30 ($O\underline{C}H_2S$), 34.75 ($\underline{C}H_2CO$), 32.76 ($S\underline{C}H_2$), 29.98 ($\underline{C}H_2CH_2CO$), 27.98 ($SCH_2\underline{C}H_2$), 25.02 ($SCH_2CH_2\underline{C}H_2$); HRMS (EI) calcd for $C_{14}H_{24}O_4S_2$ 320.1116, found 320.1128; (CI, NH_3) *m/z* (relative intensity) 338 (MNH_4^+, 100) 321 (MH^+, 85) .

For trimer **101** (7%): IR ($CHCl_3$ cast) 2933 (m), 1737 (s), 1127 (m) cm^{-1}; ^{1}H NMR (360 MHz, CD_2Cl_2) δ 5.15 (s, 6 H, 3 x $OC\underline{H}_2S$), 2.67 (t, 6 H, *J* = 7.4 Hz, 3 x $C\underline{H}_2CO$), 2.33 (t, 6 H, *J* = 7.3 Hz, 3 x $SC\underline{H}_2$), 1.72-1.60 (m, 12 H, 3 x $C\underline{H}_2CH_2CO$ + 3 x $SCH_2C\underline{H}_2$), 1.50-1.39 (m, 6 H, 3 x $SCH_2CH_2C\underline{H}_2$); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 173.24 ($\underline{C}O$), 67.17 ($O\underline{C}H_2S$), 34.55 ($\underline{C}H_2CO$), 32.79 ($S\underline{C}H_2$), 30.14 ($\underline{C}H_2CH_2CO$), 28.33 ($SCH_2\underline{C}H_2$), 24.75 ($SCH_2CH_2\underline{C}H_2$); HRMS (EI) calcd for $C_{21}H_{36}O_6S_3$ 480.1674, found 480.1668; (CI, NH_3) *m/z* (relative intensity) 498 (MNH_4^+, 100), 481 (MH^+, 21).



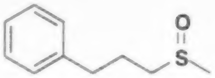
Methyl 6-(Methylsulfinyl)hexanoate (103) To a solution of **98** (1.00 g, 5.61 mmol) in CH_2Cl_2 (40 mL) cooled in an ice bath was added dropwise CH_2N_2/Et_2O solution until a pale yellow color was obtained. The mixture was stirred at room temperature for 30 min and a few drops of formic acid were added to destroy the excess CH_2N_2. Evaporation of solvent *in vacuo* gave **103** (1.07 g, 99%) as a pale yellow oil. IR ($CHCl_3$ cast) 2948 (m), 1735 (s), 1033 (m) cm^{-1}; ^{1}H NMR (400 MHz, CD_2Cl_2) δ 3.61 (s, 3 H, $OC\underline{H}_3$), 2.63 (m, 2 H, $S(O)C\underline{H}_2$), 2.48 (s, 3 H, $C\underline{H}_3S(O)$), 2.30 (t, 2 H, J = 7.4 Hz, $C\underline{H}_2CO$), 1.72 (m, 2 H, $S(O)CH_2C\underline{H}_2$), 1.64 (m, 2 H, $C\underline{H}_2CH_2CO$), 1.46 (m, 2 H, $S(O)CH_2CH_2C\underline{H}_2$); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 173.93 ($\underline{C}O$), 54.78 ($O\underline{C}H_3$), 51.61 ($S(O)\underline{C}H_2$), 38.99 ($\underline{C}H_3S(O)$), 33.91 ($\underline{C}H_2CO$), 28.54 ($S(O)CH_2C\underline{H}_2$), 24.79 ($\underline{C}H_2CH_2CO$), 22.58 ($S(O)CH_2CH_2C\underline{H}_2$); HRMS (EI) *m/z* (relative intensity) calcd for $C_8H_{16}O_3S$ 192.0820, found 192.0820 (M^+, 13). Anal. Calcd for $C_8H_{16}O_3S$: C, 49.97; H, 8.39; S, 16.67. Found: C, 50.10; H, 8.55; S, 16.50.



***endo*-Bornyl 6-Methylthiohexanoate (104)** General procedure for the modified Swern oxidation was used except that the reaction was performed at -78 °C. Thus, sulfoxide **98** (0.535 g, 3.00 mmol), triflic anhydride (0.46 mL, 2.75 mmol) and *endo*-borneol (0.386 g, 2.50 mmol) gave a dark yellow oil. Flash chromatography (2-10% Et_2O in hexane) afforded camphor (0.170 g, 45%) and **104** (0.048 g, 6%) as a colorless oil. IR ($CHCl_3$ cast) 2952 (s), 1733 (s), 1257 (m), 1189 (m) cm^{-1}; ^{1}H NMR (360 MHz, CD_2Cl_2) δ 4.86 (ddd, 1 H, J = 9.9, 3.5, 2.2 Hz, $OC\underline{H}$), 2.48 (t, 2 H, J = 7.3 Hz, $C\underline{H}_2S$), 2.37-2.28 (m, 1 H), 2.31 (t, 2 H, J = 7.4 Hz, $COC\underline{H}_2$), 2.07 (s, 3 H, $C\underline{H}_3S$), 1.95 (m, 1 H), 1.75 (m, 1 H), 1.68-1.56 (m, 5 H), 1.46-1.18 (m, 4 H), 0.95 (dd, 1 H, J = 6.8, 3.5 Hz), 0.91 (s, 3 H, $C\underline{H}_3$), 0.88 (s, 3 H, $C\underline{H}_3$), 0.82 (s, 3 H, $C\underline{H}_3$); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 173.97 ($\underline{C}O$), 79.82 ($O\underline{C}H$), 49.02, 48.07, 45.37, 37.15, 34.83 ($\underline{C}H_2S$), 34.34 ($CO\underline{C}H_2$), 29.23 ($SCH_2\underline{C}H_2$), 28.63 ($\underline{C}H_2CH_2CO$), 28.31, 27.45, 25.11 ($SCH_2CH_2C\underline{H}_2$), 19.81 ($\underline{C}H_3$), 18.97 ($\underline{C}H_3$), 15.55 (S$\underline{C}H_3$), 13.62 ($\underline{C}H_3$); HRMS (EI) *m/z* (relative intensity) calcd for $C_{17}H_{30}O_2S$ 298.1967, found 298.1966 (M^+, 10), 145.0688 (50), 137.1332 (100).

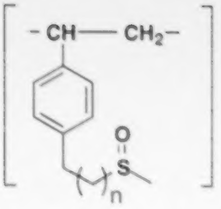
Low Temperature ^{13}C NMR Analysis. Spectra were acquired on a Bruker AM200 spectrometer with a 20 mm probe at -60 °C. CD_2Cl_2 was dried by distillation from CaH_2 before use. The reaction was done on a 1 mmol scale in a 12 mm NMR tube cooled to -60 °C. After attachment of the apparatus to a pressure-equalizing argon line, the reagents were added *via* a syringe. Chlorosulfonium intermediate **108** was added *via* a cannula which was cooled with dry ice. After each addition the NMR tube was kept at -60 °C for 15 min with occasional shaking before the spectral analysis. The ^{13}C NMR chemical shifts (δ) for the intermediates are as follows: **98**, 176.31, 52.32, 36.92, 33.22, 27.50, 23.84, 22.13; **105**, 180.80, 65.76, 47.64, 33.25, 26.84, 24.00, 23.48; **107**, 180.77, 43.78, 33.22, 27.19, 25.93, 25.60, 23.35; **103**, 173.81, 53.47, 51.54, 38.05, 33.11, 27.66, 24.02, 22.18; **108**, 173.95, 65.78, 51.69, 47.63, 32.99, 26.92, 23.99, 23.79; **109**, 178.94, 83.64, 49.69, 34.78, 33.10, 26.83, 23.49, 22.33, 21.98.

Reaction of Dimsyl Sodium with Benzyl Chloride To DMSO (50 mL) was added NaH (60% dispersion in mineral oil, 3.60 g, 90 mmol, washed three times with pentane, vacuum dried) in small portions. The mixture was then heated at 60 °C for 1 h. The mixture was cooled to room temperature, and benzyl chloride (6.9 mL, 60 mmol) was added carefully. The dark purple solution was allowed to stir for another 20 min. The reaction was quenched by adding H_2O, the mixture was extracted three times with $CHCl_3$, and the extracts were dried (Na_2SO_4). After concentration, the residue was carefully separated with flash chromatography eluting with hexane, 10% Et_2O in hexane, EtOAc, 5% CH_3OH in EtOAc successively. The first fraction was identified as *trans*-stilbene (1.3 g). A polar fraction was obtained after washing with 5% CH_3OH in EtOAc. ^{1}H NMR spectroscopy shows a mixture of sulfoxides (3.75 g, ca 37% yield, based on an average molecular weight of methyl phenethyl sulfoxide), which were identified as **111** and **112** after further purification. Chromatographic and spectroscopic properties of **111** are identical with those of authentic sample. Spectroscopic data for **112** are listed below.



Methyl 3-Phenylpropyl Sulfoxide (**112**) IR ($CHCl_3$ cast) 2923 (m), 1454 (m), 1048 (s), 749 (m), 701 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.45-7.15 (m, 5 H, Ar$\underline{H}$), 2.87-2.63 (m, 4 H, $ArC\underline{H}_2$ + $C\underline{H}_2S(O)$), 2.57 (s, 3 H, $S(O)C\underline{H}_3$), 2.18-2.08 (m, 2 H, $ArCH_2C\underline{H}_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.12, 128.28, 128.15, 126.02 (Ar-$\underline{C}$), 53.28 ($\underline{C}H_2S(O)$), 38.09 ($S(O)\underline{C}H_3$), 34.27 ($Ar\underline{C}H_2$), 23.81 ($ArCH_2\underline{C}H_2$); HRMS (EI) *m/z* (relative intensity) calcd for $C_{10}H_{14}OS$ 182.0765, found 182.0765 (M^+, 14), 91.0548 (100); MS (FAB, Cleland) *m/z* (relative intensity) 183 (MH^+, 100).

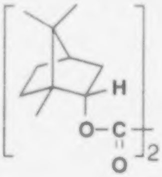
Characterization of Commercial Chloromethyl Polystyrene Resin (**113**) Commercial chloromethyl polystyrene resin (4.15 mequiv/g, 1% crosslinked, Bio-Beads S-X-1, from Bio-Rad) (161.7 g) was swollen in CH_2Cl_2 (1500 mL) overnight, then washed with CH_2Cl_2 (4 x 500 mL). After preliminary drying with an aspirator for 8 h the washed beads were further dried under high vacuum at 60 °C for 40 h. Dry resin (159.6 g) was obtained. IR (microscope) 3026 (m), 2924 (s), 2850 (m), 1603 (m), 1493 (s), 1452 (s), 1266 (s) cm^{-1}; ^{13}C NMR (100 MHz, CD_2Cl_2) δ 145.96, 128.37, 126.34, 46.88 ($Ar\underline{C}H_2Cl$), 44.35, 43.45, 40.88. Anal. Calcd, based on 4.15 mequiv/g: C, 78.68; H, 6.61; Cl, 14.71. Found: C, 77.89; H, 6.56; Cl, 15.16.



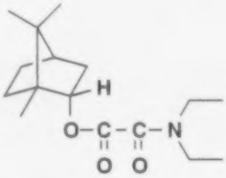
Polymer Sulfoxide Reagent (114) A solution of dimsyl sodium, prepared from NaH (7.86 g, 0.311 mol) and DMSO (200 mL), was slowly cannulated to a swollen suspension of **113** (50 g, 0.208 mol) in DMSO (800 mL). The mixture was then gently stirred for 48 h. at room temperature. A series of color changes on the beads, from pink to dark black, was observed. After the reaction had been quenched by adding water, the yellow resin was collected by filtration, then washed successively with H_2O, 1:1 CH_3OH-H_2O, CH_3OH, THF, CH_2Cl_2 (2 x 300 ml for each washing), then dried under high vacuum at 60 °C for 60 h to afford **114** (50.7 g, 87%, based on direct substitution). IR (microscope) 3024 (m), 2919 (s), 2852 (m), 1601 (m), 1493 (m), 1452 (m), 1056 (m) cm^{-1}. Anal. Calcd, based on 100% direct substitution: C, 75.64; H, 7.36; S, 11.33. Found: C, 82.47; H, 7.43; S, 5.69.

General Procedure for Polymer-Supported Swern Oxidation Polymer-supported sulfoxide **114** (2.00 g, 3.56 mmol, based on elemental analysis) was swollen in CH_2Cl_2 (20 mL) at room temperature for 15 min, and then cooled to ca -50 °C. Oxalyl chloride (1 equiv) in CH_2Cl_2 (5 mL) was added dropwise. After 1 h at that temperature, alcohol (2.67 mmol, 0.5 equiv) in CH_2Cl_2 (10 mL) was added, and the mixture was stirred for 3 h before the addition of triethylamine (3 equiv). The mixture was allowed to slowly warm to room temperature and stirred overnight. The resin was removed by filtration, and washed with CH_2Cl_2. The filtrate was concentrated to 50 mL, then diluted with Et_2O (50 mL), and washed three times with H_2O. The ethereal phases were dried (Na_2SO_4) and concentrated *in vacuo*. The product was analyzed by ^1H NMR spectrometry.

Determination of Oxidation Capacity for Polymer Sulfoxide Reagents Same procedure as the above general procedure except that one equivalent of alcohol (based on the elemental analysis of sulfur) was used. After the crude product had been analyzed by ^{1}H NMR spectrometry, the non-oxidized products were isolated and identified as **115** and **116**, which show spectroscopic data listed below.

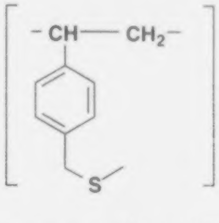


***endo*-Bornyl Oxalate (115)** IR ($CHCl_3$ cast) 2955 (s), 1768 (m), 1741 (s), 1185 (s) cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 5.03 (ddd, 2 H, *J* = 9.9, 3.3, 2.2 Hz, 2 x $OC\underline{H}$), 2.43 (m, 2 H), 2.03 (m, 2 H), 1.85-1.70 (m, 4 H), 1.45-1.25 (m, 4 H), 1.12 (dd, 2 H, *J* = 6.8, 3.5 Hz), 0.94 (s, 6 H, 2 x $C\underline{H}_3$), 0.91 (s, 6 H, 2 x $C\underline{H}_3$), 0.89 (s, 6 H, 2 x $C\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.54 ($\underline{C}O$), 82.96 ($O\underline{C}H$), 49.13, 47.95, 44.88, 36.44, 27.88, 26.98, 19.65 ($\underline{C}H_3$), 18.82 ($\underline{C}H_3$), 13.43 ($\underline{C}H_3$); HRMS (EI) *m/z* (relative intensity) calcd for $C_{22}H_{34}O_4$ 362.2457, found 362.2455 (M^+, 1.5), 181.1226 (87), 137.1317 (99).

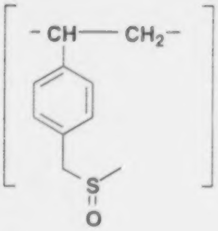


***N,N*-Diethyl Mono(*endo*-bornyl) Oxalamide (116)** IR ($CHCl_3$ cast) 2956 (m), 1735 (s), 1661 (s), 1230 (m), 1215 (s), 1127 (M) cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 5.08 (ddd, 1 H, *J* = 10.0, 3.2, 2.3 Hz, $OC\underline{H}$), 3.43 (q, 2 H, *J* = 7.2 Hz, $NC\underline{H}_2CH_3$), 3.28 (q, 2 H, *J* = 7.2 Hz, $NC\underline{H}'_2CH'_3$), 2.41 (m, 1 H), 1.96 (m, 1 H), 1.83-1.68 (m, 2 H), 1.40-1.20 (m, 2 H), 1.22 (t, 3 H, *J* = 7.2 Hz, $NCH_2C\underline{H}_3$), 1.19 (t, 3 H, *J* = 7.2 Hz, $NCH'_2C\underline{H}'_3$), 1.10 (dd, 1 H, *J* = 6.8, 3.5 Hz), 0.92 (s, 3 H, $C\underline{H}_3$), 0.88 (s, 3 H, $C\underline{H}_3$), 0.88 (s, 3 H, $C\underline{H}_3$); ^{13}C NMR (75

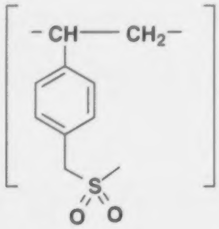
MHz, $\mathrm{CDCl_3}$) δ 163.89, 161.72 ($\mathrm{\underline{C}OO}$, $\mathrm{\underline{C}ON}$), 82.03 ($\mathrm{O\underline{C}H}$), 48.97, 48.03, 44.79, 42.21 ($\mathrm{N\underline{C}H_2CH_3}$), 38.72 ($\mathrm{N\underline{C}H'_2CH'_3}$), 36.35, 27.91, 27.00, 19.63 ($\mathrm{\underline{C}H_3}$), 18.77 ($\mathrm{\underline{C}H_3}$), 14.27 ($\mathrm{NCH_2\underline{C}H_3}$), 13.48 ($\mathrm{\underline{C}H_3}$), 12.54 ($\mathrm{NCH'_2\underline{C}H'_3}$); HRMS (EI) *m/z* (relative intensity) 137.1330 (100); MS (CI, $\mathrm{NH_3}$) *m/z* (relative intensity) 299 ($\mathrm{MNH_4^+}$, 4), 282 ($\mathrm{MH^+}$, 100).



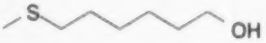
Polymer Sulfide Reagent (118) To a suspension of NaH (60% dispersion in mineral oil, 2.49 g, 62.3 mmol, pre-washed with hexane) in THF (40 mL), was bubbled methanethiol gas until the solution was saturated. The thiomethoxide solution was then cannulated to **113** (10.0 g, 41.5 mmol) swollen in THF (150 mL), and the mixture was gently stirred at room temperature over night. After acidification the excess methanethiol was removed by bubbling argon through the solution, and trapped with $\mathrm{Pb(OAc)_2}$ solution. The resin was washed successively with $\mathrm{H_2O}$, 1:1 $\mathrm{CH_3OH}$-$\mathrm{H_2O}$, $\mathrm{CH_3OH}$, THF, $\mathrm{CH_2Cl_2}$ (2 x 150 ml for each washing), then dried under high vacuum at 60 °C for 60 h to afford **118** (9.88 g, 94%). IR (microscope) 3024 (m), 2916 (s), 2849 (m), 1602 (m), 1493 (m), 1451 (s), 703 (m) $\mathrm{cm^{-1}}$; ^{13}C NMR (75 MHz, THF-d_8) δ 146.04, 128.76, 127.25, 41.38, 38.76 ($\mathrm{\underline{C}H_2S}$), 15.07 ($\mathrm{S\underline{C}H_3}$). Anal. Calcd, based on 100% substitution: C, 79.81; H, 7.50; S, 12.67. Found: C, 78.78; H, 7.51; S, 12.72.



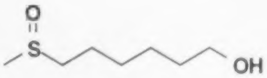
Polymer Sulfoxide Reagent (119) To a suspension of resin **113** (40.0 g, 0.159 mol) in CH_2Cl_2 (800 mL) at 0 °C was added dropwise a solution of MCPBA (50-60%, 50.0 g, 0.159 mmol) in CH_2Cl_2 (800 mL). The mixture was kept at 0 °C for another 3 h, the warmed to room temperature and stirred overnight. After filtration the resin was washed successively with H_2O, 1% $NaHCO_3$, H_2O, CH_3OH, CH_2Cl_2 (2 x 400 ml for each washing), then dried under high vacuum at 60 °C for 60 h to afford **119** (43.1 g, theoretic 42.7 g). IR (microscope) 3439 (br, m), 3025 (s), 2923 (s), 1603 (m), 1492 (m), 1452 (s), 1309 (s), 1054 (m) cm^{-1}; ^{13}C NMR (75 MHz, CD_2Cl_2) δ 127.96, 60.82 ($\underline{C}H_2S(O)$), 40.88, 37.92 ($S(O)\underline{C}H_3$). Anal. Calcd. based on 100% conversion: C, 74.75; H, 7.10; S, 10.71. Found: C, 73.46; H, 6.85; S, 11.01.



Polymer Sulfone Reagent (120) Same as above except three equivalents of MCPBA were used. Thus, resin **113** (0.20 g, 0.80 mmol) with MCPBA (80-85%, 0.515 g, 3.39 mmol) gave **120** (0.24 g, theoretical, 0.23 g). IR (microscope) 3026 (m), 2924 (s), 1452 (m), 1320 (s), 1129 (s), 966 (m) cm^{-1}; ^{13}C NMR (75 MHz, CD_2Cl_2) δ 61.24 ($\underline{C}H_2S(O)_2$), 41.02, 39.58 ($S(O)_2\underline{C}H_3$).

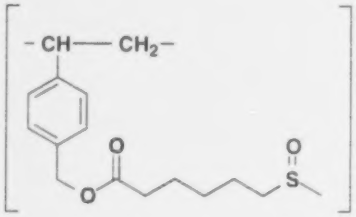


6-(Methylthio)hexanol (123) To a suspension of $LiAlH_4$ (0.250 g, 6.59 mmol) in THF (20 mL) cooled to 0 °C was added dropwise a solution of **97** (1.00 g, 6.17 mmol) in THF (10 mL), the mixture was then stirred at room temperature overnight. Water was carefully added to destroy the excess hydride. After acidification with 2N HCl, the solution was extracted with Et_2O (3 x 50 mL). Concentration of the dried ether layers (Na_2SO_4) gave **123** (0.906 g, 99%) as a colorless oil. IR ($CHCl_3$, cast) 3347 (br m), 2931 (s), 2857 (s), 1054 (m) cm^{-1}; ^{1}H NMR (360 MHz, CD_2Cl_2) δ 3.58 (t, 2 H, *J* = 6.6 Hz, $C\underline{H}_2O$), 2.47 (t, 2 H, *J* = 7.4 Hz, $SC\underline{H}_2$), 2.07 (s, 3 H, $C\underline{H}_3S$), 1.65-1.49 (m, 5 H, $SCH_2C\underline{H}_2$ + $C\underline{H}_2CH_2O$ + $O\underline{H}$), 1.46-1.30 (m, 4 H, $SCH_2CH_2C\underline{H}_2C\underline{H}_2$); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 62.97 ($\underline{C}H_2O$), 34.44 ($SC\underline{H}_2$), 33.08 ($\underline{C}H_2CH_2O$), 29.50 ($SCH_2\underline{C}H_2$), 28.93 ($\underline{C}H_2CH_2CH_2O$), 25.75 ($SCH_2CH_2\underline{C}H_2$), 15.53 ($\underline{C}H_3S$); HRMS (EI) calcd for $C_7H_{16}OS$ 148.0921, found 148.0920; MS (FAB, Cleland) *m/z* (relative intensity) 149 (MH^+, 100). Anal. Calcd for $C_7H_{16}OS$: C, 56.71; H, 10.88; S, 21.62. Found: C, 56.91; H, 11.10; S, 21.61.

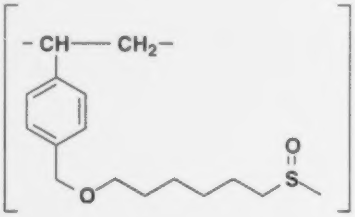


6-(Methylsulfinyl)hexanol (124) The procedure for the conversion of **97** to **98** was used. Thus, **123** (2.04 g, 13.8 mmol) with sodium metaperiodate (0.5 M, 28.9 mL, 14.5 mmol) in methanol (40 mL) gave **124** (2.29 g, 100%). (IR ($CHCl_3$ cast) 3383 (br s), 2931 (s), 2859 (m), 1024 (s) cm^{-1}; ^{1}H NMR (360 MHz, CD_2Cl_2) δ 3.53 (t, 2 H, *J* = 6.5 Hz, $C\underline{H}_2O$), 2.74 (br, 1 H, $O\underline{H}$), 2.74-2.58 (m, 2 H, $S(O)C\underline{H}_2$), 2.50 (s, 3 H, $C\underline{H}_3S(O)$), 1.72 (m, 2 H, $C\underline{H}_2CH_2O$), 1.60-1.30 (m, 6 H, $S(O)CH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2$); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 62.43 ($\underline{C}H_2OH$), 54.89 ($S(O)\underline{C}H_2$), 38.83 ($\underline{C}H_3S(O)$), 32.85 ($\underline{C}H_2CH_2O$), 28.87 ($S(O)CH_2\underline{C}H_2$), 25.77 ($\underline{C}H_2CH_2CH_2O$), 22.87 ($S(O)CH_2CH_2\underline{C}H_2$); HRMS (EI) calcd for $C_7H_{16}O_2S$ 164.0871, found 164.0870; MS (FAB, Cleland) *m/z* (relative intensity) 165

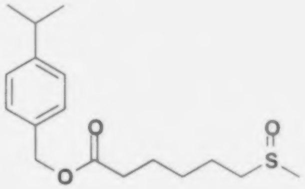
(MH$^+$, 100). Anal. Calcd for $C_7H_{16}O_2S$: C, 51.18; H, 9.82; S, 19.52. Found: C, 50.81; H, 9.97; S, 19.05.



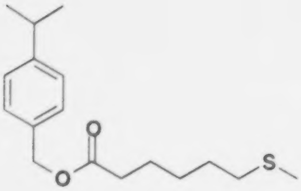
Polymer Sulfoxide Reagent (125) Sulfoxide **98** (2.95 g, 16.6 mmol) and KOH (0.93 g, 16.6 mmol) were dissolved in H_2O (20 mL). The solution was concentrated and dried under vacuum for 2 days. The resulting potassium salt was mixed with DMF (50 mL) and *p*-chloromethylated polystyrene resin (2.00 g, 8.30 mmol, Bio-Beads S-X-1, Bio-Rad, 4.15 mequiv/g). The mixture was heated at 100 °C for 20 h with a gentle stirring. After cooling, the resin was filtered, washed (MeOH, THF, CH_2Cl_2 (3 x each)), and dried at 60 °C *in vacuo* for 40 h. A white resin **125** (2.96 g, 93%) was obtained: IR (microscope) 2927 (s), 1732 (s), 1452 (m), 1258 (m), 1168 (s), 1029 (s), 703 (m) cm^{-1}; ^{13}C NMR (75 MHz, CD_2Cl_2) δ 173.33 ($\underline{C}O$), 128.51 (Ar), 66.34 (Ar$\underline{C}H_2$), 54.83 ($\underline{C}H_2S(O)$), 40.87, 39.12 ($S(O)\underline{C}H_3$), 34.23 ($C(O)\underline{C}H_2$), 28.67 ($\underline{C}H_2CH_2S(O)$), 24.91 ($C(O)CH_2\underline{C}H_2$), 22.70 ($C(O)CH_2CH_2\underline{C}H_2$). Anal. Calcd, based on 100% conversion: C, 71.12; H, 8.07; S, 8.33. Found: C, 68.38; H, 7.34; S, 7.95.



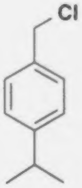
Polymer Sulfoxide Reagent (126) To a suspension of NaH (95%, 0.210 g, 8.33 mmol) in THF (20 mL) was added **124** (1.36 g, 8.30 mmol) in THF (15 mL). The mixture was heated at 45 °C for 1 h to complete the alkoxide formation. Resin **113** was then added, and the mixture was gently stirred under reflux for 72 h. The reaction was quenched with CH_3OH, the resin was collected by filtration, and washed with CH_3OH-H_2O, THF-H_2O, THF and CH_2Cl_2, then dried under high vacuum at 60 °C to afford **126** (1.45 g, 95%). IR (microscope) 2938 (s), 2986 (s), 1492 (m), 1452 (s), 1362 (m), 1113 (s), 1054 (s), 703 (m) cm^{-1}. Anal. Calcd, based on 100% conversion: C, 74.21; H, 8.42; S, 8.69. Found: C, 72.87; H, 8.69; S, 8.20.



***p*-Isopropylbenzyl 6-(Methylsulfinyl)hexanoate (127)** The procedure for the preparation of **125** was adopted. Thus, **130** (3.37 g, 20 mmol) coupled with **98** (3.92 g, 22 mmol) to form **127** (6.07 g, 98%). IR ($CHCl_3$ cast) 2959 (s), 2868 (m), 1733 (s), 1168 (s), 1036 (s), 820 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.25 (AB, 4 H, Ar$\underline{H}$), 5.08 (s, 2 H, ArC$\underline{H}_2$O), 2.92 (m, 1 H, J = 6.9 Hz, C$\underline{H}$(CH_3)$_2$), 2.75-2.60 (m, 2 H, C$\underline{H}_2$S(O)), 2.55 (s, 2 H, S(O)C$\underline{H}_3$), 2.37 (t, 2 H, J = 7.4 Hz, C(O)C$\underline{H}_2$), 1.78 (m, 2 H, C$\underline{H}_2$$CH_2$S(O)), 1.70 (m, 2 H, C(O)CH_2C$\underline{H}_2$), 1.59-1.39 (m, 2 H, C(O)CH_2CH_2C$\underline{H}_2$), 1.25 (d, 6 H, J = 6.9 Hz, CH(C$\underline{H}_3$)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.08 ($\underline{C}$O), 148.96, 133.22, 128.31, 126.53 (Ar), 66.06 (Ar$\underline{C}H_2$O), 54.29 ($\underline{C}H_2$S(O)), 38.49 (S(O)$\underline{C}H_3$), 33.81 (C(O)$\underline{C}H_2$), 33.77 ($\underline{C}$H(CH_3)$_2$), 28.10 ($\underline{C}H_2CH_2$S(O)), 24.34 (C(O)$CH_2\underline{C}H_2$), 23.84 (CH($\underline{C}H_3$)$_2$), 22.16 (C(O)$CH_2CH_2\underline{C}H_2$); HRMS (EI) *m/z* (relative intensity) calcd for $C_{17}H_{26}O_3S$ 310.1603, found 310.1599 (M^+, 0.3), 179.0737 (29) 161.0632 (30), 133.1011 (100); MS (FAB, Cleland) *m/z* (relative intensity) 311 (MH^+, 100). Anal. Calcd for $C_{17}H_{26}O_3S$: C, 65.77; H, 8.44. Found: C, 65.63; H, 8.53.



_p_-Isopropylbenzyl 6-(Methylthio)hexanoate (128) The procedure for the preparation of **125** was followed. Thus, **130** (1.69 g, 10 mmol) coupled with **97** (1.79 g, 11 mmol) to form **128** (2.75 g, 94%) after purification by flash chromatography (10% Et_2O in hexane). IR ($CHCl_3$ cast) 2959 (m), 1736 (s), 1166 (m), 819 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.25 (AB, 4 H, $\mathrm{Ar\underline{H}}$), 5.08 (s, 2 H, $\mathrm{ArC\underline{H}_2O}$), 2.91 (m, 1 H, $J = 6.9$ Hz, $\mathrm{C\underline{H}(CH_3)_2}$), 2.47 (t, 2 H, $J = 7.3$ Hz, $\mathrm{C\underline{H}_2S}$), 2.35 (t, 2 H, $J = 7.5$ Hz, $\mathrm{C(O)C\underline{H}_2}$), 2.07 (s, 3 H, $\mathrm{SC\underline{H}_3}$), 1.66 (m, 2 H, $\mathrm{C(O)CH_2C\underline{H}_2}$), 1.60 (m, 2 H, $\mathrm{C\underline{H}_2CH_2S}$), 1.41 (m, 2 H, $\mathrm{C(O)CH_2CH_2C\underline{H}_2}$), 1.25 (d, 6 H, $J = 6.9$ Hz, $\mathrm{CH(C\underline{H}_3)_2}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.38 ($\mathrm{\underline{C}O}$), 148.92, 133.34, 128.33, 126.54 (Ar), 66.01 ($\mathrm{Ar\underline{C}H_2O}$), 34.09 ($\mathrm{\underline{C}H_2S}$), 33.93 ($\mathrm{C(O)\underline{C}H_2}$), 33.80 ($\mathrm{\underline{C}H(CH_3)_2}$), 28.68 ($\mathrm{\underline{C}H_2CH_2S}$), 28.15 ($\mathrm{C(O)CH_2\underline{C}H_2}$), 24.47 ($\mathrm{C(O)CH_2CH_2\underline{C}H_2}$), 23.87 ($\mathrm{CH(\underline{C}H_3)_2}$), 15.40 ($\mathrm{S\underline{C}H_3}$); HRMS (EI) *m/z* (relative intensity) calcd for $C_{17}H_{26}O_2S$ 294.1653, found 294.1647 (M^+, 2), 161.0631 (37) 143.0518 (68), 133.1012 (100). Anal. Calcd for $C_8H_{16}O_2S$: C, 69.34; H, 8.90; S, 10.89. Found: C, 69.66; H, 9.19; S, 10.65.



_p_-Isopropylbenzyl Chloride (130) To a solution of **129** (9.01 g, 60.0 mmol) in CH_2Cl_2 (100 mL) was added triphenylphosphine (18.88 g, 72.0 mmol) in CCl_4 (50 mL). The mixture was then stirred at room temperature overnight. After the removal of solvent *in vacuo* the residue was filtered through a silica gel column, washed with 15% Et_2O in hexane. Concentration of the filtrate gave **130** (8.58 g, 85%) as a colorless oil. IR ($CHCl_3$ cast) 2961 (s), 1265 (m), 836 (m), 674 (m) cm^{-1}; ^1H NMR (360 MHz, CD_2Cl_2) δ 7.29

(AB, 4 H, $\mathrm{Ar\underline{H}}$), 4.60 (s, 2 H, $\mathrm{ArC\underline{H}_2Cl}$), 2.93 (m, 1 H, J = 6.9 Hz, $\mathrm{C\underline{H}(CH_3)_2}$), 1.26 (d, 6 H, J = 6.9 Hz, $\mathrm{CH(C\underline{H}_3)_2}$); ^{13}C NMR (100 MHz, $\mathrm{CD_2Cl_2}$) δ 149.82, 135.46, 129.04, 127.19 (Ar), 44.77 ($\mathrm{Ar\underline{C}H_2Cl}$), 34.30 ($\mathrm{\underline{C}H(CH_3)_2}$), 24.07 ($\mathrm{CH(\underline{C}H_3)_2}$); HRMS (EI) *m/z* (relative intensity) calcd for $\mathrm{C_{10}H_{13}{}^{37}Cl}$ and $\mathrm{C_{10}H_{13}{}^{35}Cl}$ 170.0676 and 168.0706, found 170.0677 ($\mathrm{M^+}$, 15) and 168.0706 (47), 153.0471 (100).

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